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A technique to collect male stingless bees

Stingless bees that belong to order Hymenoptera, family Apidae and tribe Meliponini, are one of the important beneficial insects acting as pollinators and honey-yielders[1–3]. There are about 500 described species belonging to 60 genera distributed in the tropical and subtropical regions of Africa, Australia, Indo-Malaya, Central and South America with the greatest diversity in the Amazonian rain forest of the New World[3–5]. In India, only 14 species of stingless bees belonging to three genera, namely *Lepidotrigona*, *Lisotrigona* and *Tetragonula* have been reported so far[5–7]. However, Rasmussen[5] while summarizing information on the described species of stingless bees from India predicts the potential of encountering several more species.

In stingless bees, males and females are extremely similar, making it difficult to distinguish sexes without microscopic examination[3,8–10]. However, male genitalia prove to be valuable diagnostic characters for distinguishing species complexes[5,9–11]. According to Rasmussen[5], as a first step to understand the full diversity of stingless bees of the Indian subcontinent, especially '*iridipennis*' species, taxonomic revision should include morphological characters of male genitalia, and it remains premature to describe and propose additional species of *Tetragonula* until their males are discovered. Attasopa *et al.*[11] also stressed the need to collect males and use them for the description of species. However, males are poorly represented in museum collections[10], as they are seasonal and produced occasionally[3,8]. In India, only three species (*Tetragonula iridipennis* Smith, *Tetragonula fuscobalteata* Cameron and *Lisotrigona chandrai* Viraktamath and Sajan Jose) are known by both males and females, while the rest only by females. Considerable effort and time are required to collect males of stingless bees[11], as swarming in Meliponini is infrequent[12].

Methods to collect males presently include continuous monitoring of nests for emergence of mating flights, searching for congregation sites (leks) of males[13], complete destruction of the entire nest, collecting foraging bees in specimen tubes and sexing them after killing or anesthetizing. However, the last method did not yield any male in an extensive survey conducted from 2008 to 2012 in seven states of India[14]. Attasopa *et al.*[11] emphasized that researchers make considerable efforts to collect males of Meliponini without destroying the nests.

During our efforts to collect males by monitoring colonies for the emergence of mating flights, we observed many foraging bees flying downwards immediately after coming out of the nest before moving away for foraging. In colonies nesting close to the ground (30–40 cm from the ground), foraging bees often fell down on their back, and then regained their position and flew away. This interesting behaviour lead us to explore the possibility of trapping such bees that may also include males, as male bees are known to leave the nest permanently after attaining maturity in search of virgin queens[3,8,12].

This hypothesis was tested by designing a trap that could retain the fallen bees in a container, and this trap was evaluated in stingless bee colonies of *Tetragonula* species at seven different locations. We also evaluated this technique for a one-year period (August 2017 to August 2018) in three colonies of stingless bees (*Tetragonula* nr. *pagdeni*) at Bengaluru, Karnataka, India. Results of these studies are described here.

A simple trap was prepared using the lower half of 1 liter plastic drinking water bottle or a plastic cup of 200 ml capacity (Figure 1). Four holes of 2 mm diameter were made below the rim of the trap at different directions. Two holes in opposite direction were used for inserting a wire or thread for hanging the trap for installation and the other two holes served as the drain for excess water in case of rain. About 100 ml of water was poured in the trap, which was replenished as and when required throughout the study period.

Water traps were evaluated in seven diverse locations, namely Bengaluru, Dharwad and Mankalale (Karnataka), Gandacherra (Tripura), Medziphema (Nagaland), Thawai (Manipur) and Paralakhemundi (Odisha). The traps were positioned 5–10 cm below the entrance of stingless bee colonies of *Tetragonula* species with the help of thread or iron wire for 4–15 days during different periods (Table 1). Bees trapped were collected daily at Bengaluru and Dharwad, and on alternate days in other places. Bees trapped were preserved in 95% alcohol vials separately and labelled. They were later examined under a stereoscopic microscope for identification of sex and counting of all the trapped bees. Males were identified based on the presence of bifid claws and genitalia. Percentage of males trapped was calculated.

Traps were evaluated for one year from August 2017 to August 2018 (53 weeks) at the GKVK Campus, Bengaluru, and the bees trapped were monitored from three colonies of stingless bees. These colonies had 15–20 foraging bees leaving the nest per minute indicating equal strength of the colonies, as the number of foraging bees depends on the strength of the colony[13]. Bees in each trap were collected in vials containing 95% alcohol daily, labelled and later examined under a stereoscopic binocular microscope in the laboratory. Workers and male bees were counted and recorded separately for each colony. Later, the data were pooled for each standard week and average number of males and workers trapped per colony was calculated. As there was wide variation in the number of trapped males and females, the data were transformed to $\log_{10}$ of $x + 1$ for analysis. Pearson's correlation was calculated to study the relationship between male and worker catches.

Our hypothesis that stingless bees trapped in water may contain males turned out to be true. Male stingless bees (*Tetragonula* spp.) were successfully collected by adopting this technique in all the seven locations evaluated. Bees trapped in water included both males and females. We were able to collect males within a day of installation of traps at Dharwad and Bengaluru, which were monitored daily. Irrespective of duration of the traps (up to 15 days) males were trapped in all locations, though their number varied from 0.27 to 2.50 per day (Table 1). Females were trapped in larger numbers (14.07 to 102.00 per day) than males. The males constituted 0.95% of total bees trapped at Gandaceherra to 4.86% at Mankalale. The overall composition of males was to the extent of 2.44% of total trap catches. Wide variation in male capture in different locations appears to be natural as the period of trapping as well as species of stingless bees in these seven locations varied. In studies where brood was sampled periodically, or males were counted by opening the nest, the males constituted 8% (ref. 15) and 17.3% (ref. 16) in *Melipona favosa*, 7.7% in *Melipona trinitatis*[16], 1.7–6.7% in *Melipona bicolor*[17], and generally 6–23% in stingless bee species[12]. The internal structure and strength of the colony in these seven locations were not ascertained, which could be the reason for wide variation in males trapped in the present study.

The traps used for an entire year at GKVK, Bengaluru, proved useful in trapping male bees. Males were successfully trapped throughout the year with intermittent gaps ranging from 1 to 7 weeks, when no males were trapped. Mean the number of males trapped per colony was the highest (7.33/colony) during the 36th standard week (3–9 September 2017), while in other weeks it ranged from 0.33 to 3.33/colony. Females were also trapped throughout the year, but without any gaps as observed in males. The mean number of females trapped per colony ranged from 5.67 to 494.67.

The peak in the number of males trapped closely followed that of female bees. Whenever female catches peaked, the male catches also followed the same trend. This was also corroborated by a significant positive correlation between male and female trap catches ($r = 0.551$). Velthuis *et al.*[12] reported that male production in a colony depends on colony strength and demographic composition. Male production increased with increase in colony strength. Though we did not open the individual colonies to ascertain their strength, similarity in the trend of male and female catches for one full year and significant positive correlation would indicate this trend.

Male production is an investment to the stingless bee colony as males are essential in reproduction of the colony. According to Velthuis *et al.*[12], in many *Melipona* species a balance in ratio between queen, workers and males is maintained in the colonies. More males will be produced depending on the need of the colony. They speculated similar situation in other species of Meliponini.

Our results may not reflect the actual trend of male production in a colony as influenced by colony cycle, nesting cycle and weather parameters, but data on male trap catches can be considered as an

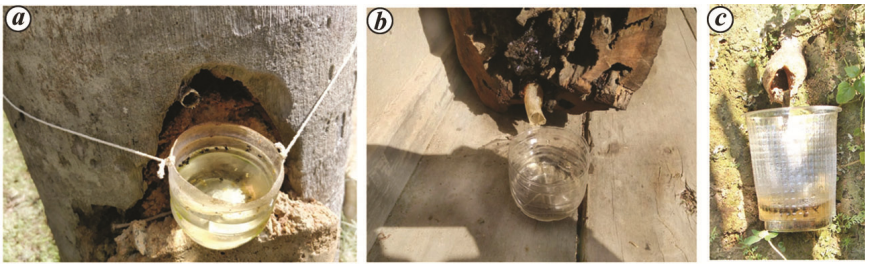

Figure 1. Colonies of *Tetragonula* species with water traps at (***a***) Bengaluru, (***b***) Thawai, (***c***) Gandacherra.

Table 1. Evaluation of water trap to collect male bees (*Tetragonula* species) at different locations

Location	Nesting place	Month of trap installation	Trapping duration (days)	Female bees trapped per colony	Male bees trapped per colony	Percentage of males
GKVK, Bengaluru, Karnataka	Wall cavities, pipes and tree cavity	August 2017	15	211 (14.07)	4 (0.27)	1.86
Dharwad, Karnataka	Cement wall cavity	September 2017	4	408 (102.00)	10 (2.50)	2.39
Mankalale, Karnataka	Wall cavity	December 2017	7	235 (33.57)	12 (1.57)	4.86
Gandacherra, Tripura	Mud wall cavity		15	521 (34.73)	5 (0.33)	0.95
Medziphema, Nagaland	Wall cavity	June 2018	10	580 (58.00)	24 (2.40)	3.97
Thawai, Manipur	Hive box	November 2017	10	572 (57.2)	9 0.90)	1.55
Paralakhemundi, Odisha	Wall cavity	December 2017	10	155 (15.50)	3 (0.3)	1.89
Total				2682	67	2.44

Bees trapped per day in parenthesis.

indication of the trend of male production. We predict higher constitution of males in this species as some foraging males may not have fallen in the trap. A periodical sampling of brood from the nests and counting emerging male bees may be required to ascertain these results.

The diversity of Indian stingless bees is still poorly known and handicapped by lack of extensive sampling of both males and females (workers and reproductives) of the species. This is particularly true for *T. iridipennis*, which requires thorough taxonomic revision. This is only possible by examining the variations in male genitalia, as suggested by Rasmussen[5]. The importance of male genitalia in resolving species complexes in stingless bees has been emphasized by Attasopa *et al.*[11].

The trapping method described here enables collection of male bees without sacrificing the colony and without the need to wait for mating flight of reproductives from a colony. This technique may help obtain male stingless bees across genera, and thereby improve our understanding of the systematics and taxonomy of this group.

However, a word of caution on the use of this water trap. Since a large number of female bees are also trapped along with males, use of the trap should be discontinued immediately after obtaining males, which may vary from 1 to 15 days. If the objective is to study male production pattern in stingless bees, this technique appears to be the best as others[15–17] are not useful for the following reasons:

(1) Most of the Indian species of stingless bees (*Tetragonula*, *Lepidotrigona* and *Lisotrigona*) have cluster type of brood and handling of brood is difficult.

(2) Females and males are similar, and it is not possible to distinguish them either in pupal or adult stages without microscopic examination (S. Viraktamath, unpublished).

(3) Probability of collecting males by capturing foraging bees in a specimen tube is nearly zero[14].

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