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Propagation of stingless bees *Tetragonula iridipennis* Smith from colonies with or without queen cell

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Abstract
Multiplication of stingless bees, *Tetragonula* spp has been a challenging task for beekeepers and scientists in India. Even though a few methods are available for increasing the colony numbers, refinement is essential to reduce the time taken and increase the success rate. Hence attempts were made in this study, to divide the stingless bee colonies using two different methods namely colony division using natural or existing queen cells and colony division by induction of emergency queen cells and compared with control colonies (colony formation through swarming) with an objective of finding out if stingless bee colonies can be produced by induction of emergency queen cells. Another experiment was conducted to find out the effective distance of placing the divided bee colonies from drone congregation site for successful mating of the newly emerged gynes. Three different distances namely placing of divided colony at 2m, 10m and 100m from the drone congregation site were studied. Based on the research, we could find that upon dividing any given colony, it is possible to get emergency queen cells from which new queens emerge, mate with drones, and start laying eggs within 70 days period. After division of a colony, initially provision of sugar solution feed is essential to maintain the growth and colony population. It was found that, the divided colonies which were placed at a distance of either 2m or 10 m from the drone congregation site, mated and started laying eggs early (at 10.88 days) and the colonies placed at 100m distance started laying eggs after 23.11 days (a delay of 12.23 days). Hence it could be concluded that the divided colonies placed within a distance of 10 m from drone congregation site ensures quick mating of the gynes.

Keywords: Stingless bees, division, Gyne, drone aggregation, mating, colony development

1. Introduction
Stingless bees are the most common bees on the planet. They are most likely the first social bees to evolve from their fewer social forefathers. In India, stingless bees are also called as dammer bees because they collect the hard resins called dammer from different trees of tropical and sub-tropical areas (Rasmussen, 2013) [6]. These bees belong to the order Hymenoptera: superfamily Apoidae, family Apidae, and subfamily Meliponinae. Meliponinae is made up of two genera, Melipona and *Trigona*, which are both members of the Meliponini and Trigonini tribes. Meliponinae is made up of eight genera, 15 subgenera, and about 500 species (Wille, 1983) [10]. They are extremely sociable insects that live in permanent colonies, nesting in old walls, logs, crevices, and other hidden locations, like honeybees. The collected pollen and honey are stored in pots. Since the sting of stingless bees is much reduced and modified and not effective for stinging, they use their mandibles to defend their colony from invaders (Michner 2000). Queens of the *Trigona* genera are grown in specially created royal cells with copious amounts of same quality food, mostly twice or thrice that of the worker cell, showing the trophic method of caste determination. In stingless bees the number of gynes (virgin queens) produced depends on the species, colony population, available food source, physiological condition of the physogastric queen, and the colony's reproductive cycle (Engels 1990) [1]. In recent days, the number of people interested in meliponiculture has been gaining momentum due to the potential of stingless bees in crop pollination, medicinal honey production, and recreational beekeeping. Hence, the colonies must be multiplied rapidly to meet the emerging demand. A lot of research work on colony production has been conducted pertaining to Melipona (Van Veen *et al*., 1999) [9]. However, no standardized methods are available for the multiplication of the colonies of *Tetragonula iridipennis* the most common species in Tamil Nadu. Beekeepers, divide the stingless bee colonies when they produce a queen cell under high population (which is referred to in this article as natural queen cell).

But in order to divide a colony, one has to wait for the natural queen cell which may take time to occur naturally. Hence in the current study, instead of waiting for the natural occurrence of queen cells, the colonies were divided even without natural or existing queen cells, with an objective of inducing the production of emergency queen cells as in honeybees (*Apis* sp.). Studies were also conducted to find out the effective distance of placing the divided bee colonies from drone congregation site for successful mating of the newly emerged gynes.

2. Materials and Methods

2.1. Stingless bees used for study

This study was conducted in Tamil Nadu Agricultural University, Department of Entomology, Insectary Unit (11°. 01' N/ 76°.92' E) during 2021-2022. In the present study, various methods of colony division in stingless bees *Tetragonula iridipennis* were compared. Stingless bee colonies were kept in horizontal rectangular wooden boxes and covered with transparent plastic sheet that enabled the close observation of the activities of stingless bees.

2.2 Colony division

For division, strong queen right colonies with dense population of bees, fully developed brood area, and copious amounts of pollen and honey pots were chosen. The boxes were cleaned, and resin was smeared around the entrance hole to attract worker bees which also prevents natural enemies from getting inside the hive. Colony division was attempted in stingless bees using two different methods.

In the first method, strong colonies having one or two queen cells was divided. Care was taken to ensure that the queen cells were transferred to the queenless division of the colony while the other part of the colony had the queen.

In the second method, strong colonies without any naturally formed queen cells were divided. This method of division induced the formation of emergency queen cells in the queenless part of the division.

These two methods of division and the performance of the daughter colonies were compared with that of the stingless bee colonies that multiplied through natural swarms (control colonies - without manual division).

Honey solution feeding was given to the divided colonies in the ratio of honey and water at 75:25 at weekly intervals. This ensured the quick development of the divided colonies. Statistical analysis, namely the analysis of variance (ANOVA), was performed on the data. Individual treatments' statistical significance was determined using a factorial completely randomised block design (FCRD) at a $P<0.05$ level.

2.3 Determining the suitable hive distance from drone congregation site

The drone congregation behaviour was observed in *Tetragonula iridipennis* which helps in the easy mating of the newly emerged gynes. Observations on drone congregation was recorded at 15-minute intervals for six days, from 9 a.m. to 5 p.m. The divided colonies were placed at different distances, namely 2, 10, and 100 m of the known drone congregation site and observed for the mating of newly emerged gynes. Statistical analysis, such as analysis of variance (ANOVA), was performed on the data. Individual treatments' statistical significance was determined using a factorial completely randomised block design (FCRD) at a $P<0.05$ level.

3. Results

The observations recorded on various methods of colony division and their effect on colony performance indicated that the number natural / emergency queen cells formed were on par in T1 -Colony division using natural queen cells (1.43) and in T2 -Colony divided to produce emergency queen cells (1.14). The number of gynes emerged at 50 Days (1.00 each) and the volume of brood cells with eggs after 60 days (118.4 and 123.3 cm3) were also on par in these two methods (Table 1). The brood volume, the pollen pot volume and the honey pot volume after 60 days were 274.9, 137.3 and 38.3 cm3 (Table 2) in T1 - Colony division using natural queen cells. In T2 -Colony divided to produce emergency queen cells, these values were 288.9, 161.8 and 57.0 cm3 which were on par with T1. In the T3-control colonies (colonies formed by natural swarming), these values were significantly lower (264.0, 135.1 and 36.6 cm3).

With this finding, we could understand and establish that colony division can be done in stingless bees even in a colony that does not have natural queen cells. Upon dividing any given colony, it is possible to get emergency queen cells from which new queens emerge and start egg laying after mating.

Nunes (2015) reported in *Tetragonula carbonaria* that colony propagation is based on the division of one colony into two smaller colonies. The process generates a queenless colony and requires that the new colony is successful in the production of a new queen (Emergency queen). In the present study too, emergency queen cells were produced. Heard (1988) [2] reported in *Tetragonula carbonaria* that colony division was successful with substantial increase in weight of the brood when divided in spring and summer than in autumn and winter in Australia. In the present study, we could achieve successful colony division of T. iridipennis during the months of Jan to April 2022 that coincides with end of winter and the entire spring season in Southern India. Mounica (2019) reported that in T. iridipennis colonies divided with natural queen cell established successfully after the gyne emergence and started egg-laying. In the present study, it has been established that the colony divided with natural queen cell as well as those without natural queen cell established successfully and the success in the latter could be attributed to the formation of emergency queen cell.

In another study by Mythri (2018) [5] with T. iridipennis, it was reported that the colonies divided without a natural (or existing) queen cell has a zero percent success rate. However, in the current study, it has been observed that the colony divided without an existing or natural queen cell also can establish successfully by forming an emergency queen cell.

In the present study, it was observed that the total period taken was about 70 days for a queen to emerge and start laying eggs. The days taken for queen mating was not significantly different among the different methods of hive division. The mean number of days taken for queen mating was 15.2, 14.8 and 14.9 (Table 3) in the different methods of colony division and were on par with one another. However, there were significant differences in the days taken for queen mating when the divided colony was placed at different distances from the drone congregation site. The days taken for queen mating were 11.3 and 10.4 in the divided stingless bee colonies when the drone congregation site was at 2 and 10 m from the hives and were on par with each other. The number of days taken for queen mating was 23.1 in colonies located at a longer distance of 100 m from the drone congregation site and was significantly different from those located at 2 and 10 m. In the present study a drone congregation site was noticed on the trunk of a tree in the meliponiary which was a non-nest associated drone congregation site. Thus, it could be concluded that a divided stingless bee colony can be placed at the distance of upto 10 m from a known / observed drone congregation site in the meliponiary for easy mating of the newly emerged gyne which increases the success rate of queen mating in the divided colonies

Stingless bee species (Melipona), males congregate in non-nest associated congregation sites such as concrete wall or cabin which are periodically visited by workers and queens (Sommeijer & de Bruijn, 1995) [8]. Santos *et al*. (2014) [7] reported from Brazil that in a meliponiary located in the city of Mossoró, state of Rio Grande do Norte, for one day in March 2009. *Melipona subnitida* (Ducke) males gathered on a branch of a cashew tree Anacardium occidentale (Anacardiaceae). This congregation occurred in a meliponiary containing approximately 90 hives of this species was observed between 10:00 and 17:00h. The males were either sitting in the congregation site or flying close to it. In the present study, the drone congregation was observed in the trunk / bark of a tree, Peltophorum pterocarpum commonly known as Copperpod at a height of about 3 feet from the ground.

Table 1: Effect of colony division method on the queen production and egg laying in stingless bees at TNAU Apiary during spring 2022 (Jan- March 2022)

	Treatments	No. of spare/ emergency queen cells produced	No. of gynes emerged (after 50 Days)	Volume of brood cells with egg (cm^3) (after 60 days)
T1	Colony division using spare queen cells produced	1.43 (1.38) a	1.00 (1.20) a	118.429 ab
T2	Colony division through emergency queen cells produced	1.14 (1.28) a	1.00 (1.20) a	123.271 a
T3	Control colonies (no. of swarm colonies produced)	0.43 (0.93) b	0.29 (0.86) b	112.014 b
	CD (0.05)	0.23	0.29	6.32

Table 2: Colony performances in the stingless bee colonies produced by various methods of colony division at TNAU Apiary during spring 2022 (Jan- March 2022)

	Treatments	Brood volume (after 60 days)	Pollen pot volume (after 60 days)	Honey pot volume (after 60 days)
T1	Colony division using spare queen cells produced	274.9 ab	137.3 ab	38.3 ab
T2	Colony division through emergency queen cells produced	288.9 a	161.8 a	57.0 a
T3	Control colonies (no. of swarm colonies produced)	264.0 b	135.1 b	36.6 b
	CD (0.05)	19.3	18.4	14.6

Table 3: Effect of distance between the divided colony and drone congregation area on the days taken for queen mating in different methods of hive division

	Treatments (Different methods of hive division)	Days taken for queen mating			
		Distances between the divided colony and drone congregation area			
		2m	10 m	100 m	Mean
T1	Colony division using spare queen cells produced	11.33 a	10.00 a	24.3 b	15.2
T2	Colony division through emergency queen cells produced	11.67 a	10.33 a	22.3 b	14.8
T3	Control colonies (no. of swarm colonies produced)	11.00 a	11.00 a	22.7 b	14.9
		11.33 a	10.44 a	23.11 b	
	T CD (0.05)	ns			
	P CD (0.05)	1.75			
	T x P CD (0.05)	3.03			

Fig 1.1: Natural or existing Queen cell after division **Fig 1.2:** Emergence Queen cell produced after division

Fig 1: Natural or existing and Emergency queen cells produced


Fig 2.1: Gyne emerged after division in T1 **Fig 2.1:** Gyne emerged after division in T2

Fig 2: Gynes emerged


Fig 3.1: Brood volume in T1 after 50 days **Fig 3.2**: Brood volume in T2 after 50 days

Fig 3: Brood volume


Fig 4.1: Pollen pot volume in T1 after 50 **Fig 4.2:** Pollen pot volume in T2 after 50 days

Fig 4: Pollen pot volume


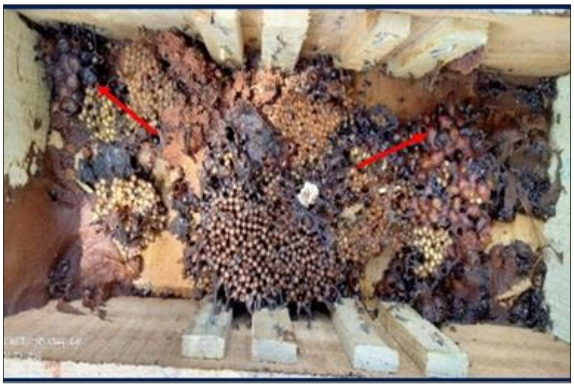

Fig 5.1: Honey pot volume in after 50 days

Fig 5: Honey pot volume

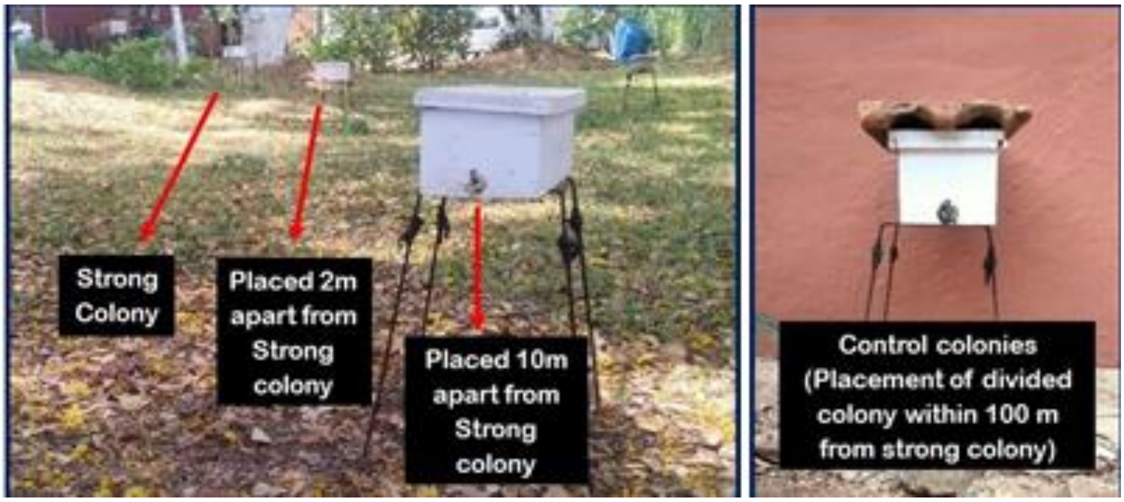

Fig 6: Divide colonies were placed near the drone aggregation colony

4. Conclusion

In the stingless bees *T. iridipennis*, the usual practice is to divide colonies having existing or natural queen cells. In the present study, it has been found that colony division can be done in *T. iridipennis* even in a colony that does not have natural queen cells. Upon dividing any given colony, it is possible to get emergency queen cells from which new queens emerge and start egg laying after mating. Secondly, a divided stingless bee colony can be placed within a distance of 10 m from a known / observed drone congregation site in the meliponiary for successful mating of the newly emerged gyne.

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