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Colony multiplication in stingless bee, *Tetragonula iridipennis* Smith using artificial division methods

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Abstract

Meliponiculture, the rearing of stingless bees is important for pollination and hive products, but limited colony availability restricts large scale practices due to slow and seasonal natural swarming. Therefore, the present study was conducted to identify a suitable method of colony division for *Tetragonula iridipennis* Smith to facilitate sustainable colony multiplication by six different methods. A total of 18 colonies were divided and the success of each method was assessed based on new queen development and survival of divided colonies. Among the six methods, separation of brood with gynes showed the highest success rate (100%), followed by separation of brood with queen cells and parallel divisions with gynes or queen cells (66.66%). Colony divisions without gynes or queen cells had lower success (33.33%), while the budding method failed to establish new colonies. The results indicate that the presence of gynes or queen cells is crucial for successful colony establishment and colony division is most effective during periods of natural gyne or queen cell development.

Keywords: Gynes, meliponiculture, queen cell and stingless be

Introduction

Stingless bees (Meliponinae) are eusocial insects belonging to the family Apidae and subfamily Meliponinae, widely distributed across tropical and subtropical regions of the Afrotropical, Australasian, Indo-Malayan and Neotropical zones with highest diversity in the Amazon rainforest (Heard, 1999; Rasmussen, 2017) ^[4, 12]. In India, they are commonly called dammer bees due to their collection of resinous substances known as dammer from various trees in tropical and subtropical areas (Rasmussen, 2017) ^[12]. *Tetragonula iridipennis* Smith, the most widespread and robust stingless bee in Karnataka, is well-suited for meliponiculture practices (Mythri *et al.*, 2018) ^[9].

These bees are highly social, nesting in permanent cavities such as tree trunks, old walls, crevices and termite mounds, where pollen and honey are stored in pots (Michener, 2000; Wille, 1983). Unlike honey bees, stingless bees possess a reduced sting and rely on mandibles for colony defence. Queens in the Trigona genus are reared in specially constructed royal cells with mass provisioning, illustrating the trophic caste-determination method. The number of gynes (virgin queens) produced depends on species, colony population, available food, physiological condition of the physogastric queen and colony reproductive cycle (Engels and Imperatriz-Fonseca, 1990) ^[1]. Newly emerged gynes perform nuptial flights, often from distances dictated by proximity to drone congregation sites, before establishing new colonies (Mythri *et al.*, 2018) ^[9].

Stingless bees are vital pollinators of cultivated and wild plants and produce honey with medicinal properties, which commands a higher market price compared to honeybee honey (Heard, 1999) ^[4]. However, natural nests are often difficult to harvest without destroying a large portion of the colony and thousands of nests are lost each year due to deforestation or urbanization (Slaa *et al.*, 2006) ^[14]. The natural swarming in stingless bee is a slow and seasonal process that limits rapid expansion of colonies (Jailani *et al.*, 2019) ^[5]. So, limited availability of colonies is a major constraint for expanding meliponiculture. Therefore, commercial beekeepers depend on wild colonies to expand their stocks. The conventional practice of hiving wild colonies typically involves dismantling the natural nest and transferring its components-brood cells, storage pots and foragers into artificial hives.

This approach however, results in the complete destruction of long established perennial colonies, leading to substantial annual losses of wild nests (Heard, 1988) [3].

Sustainable colony multiplication through standardized division technique provides a suitable alternative to wild colony harvesting (Pradeep *et al.*, 2022) [10]. Colony propagation not only reduces pressure on wild populations but also facilitates targeted placement of colonies for pollination services (Heard, 1999) [4]. However, successful division requires technical knowledge of right timing, suitable donor colony selection and species-specific behaviour (Jailani *et al.*, 2019) [5]. Inappropriate handling can lead to colony failure. Therefore, standardizing a low-risk, reproducible colony division method is essential for sustainable meliponiculture and for meeting the rising demand for stingless bee colonies. Hence the present investigation was aimed to study the best method of colony division in order to increase their numbers.

In this context, the present study was undertaken with an objectives:

1. To standardize colony division techniques in stingless bees

Material and Methods

The present investigation was carried out at the Centre of Excellence in Apiculture, College of Agriculture, Gangavathi, University of Agricultural Sciences, Raichur, Karnataka, India during March-April 2025. A total of 18 colonies were chosen and divided using six different techniques, with three colonies for each method (explained below) and the success rate (i.e. Development of queen and continuation of the divided part) was worked out in each of the method.

Method 1: Parallel division with presence of gynes in the divided part

The colonies maintained in hives made up of two rectangular halves, were selected. Colonies were regularly inspected for the emergence of gynes. Once gynes were observed, the colonies were divided into two equal parts, by separating the two halves with the brood and bees in them. Each of the separated part was provided with similar sized empty halves to make the hive a complete set of two halves. Once they were separated, both of them (part having the parent queen and the part having the gynes) were placed parallel to each other within a distance of five cm (Fig. 1).

Method 2: Parallel division with presence of queen cells in the divided part

The colonies maintained in hives made up of two rectangular halves, were selected, colonies were regularly monitored for the formation of queen cells. When queen cells were observed in colonies, then colonies were divided into two equal parts, by separating the two halves with the brood and bees in them. Each of the separated part was provided with similar sized empty halves to make the hive a complete set of two halves. Once they were separated, both of them (Part having the parent queen and the part having the queen cells) were placed parallel to each other within a distance of five cm (Fig. 1).

Method 3: Separation of brood with presence of gynes in the divided colony

Well-developed colonies in which there was presence of gynes were selected, brood (approximately volume of about 200 to 250 cm³) along with the worker bees and gynes were

taken out and placed into a new hive. Then new hive was placed separately away from the parent colony, at a distance of about 5 m (Fig. 2).

Method 4: Separation of brood with presence of queen cells in the divided colony

Well-developed colonies in which there was presence of queen cells were selected, brood (approximate volume of about 200 to 250 cm³) along with the worker bees and queen cells were taken out and placed in a new hive. Then new hive was placed separately away from the parent colony, at a distance of about 5 m (Fig. 2).

Method 5: Separation of brood in absence of gynes or queen cells in the divided colony

Well-developed colonies were selected, brood (approximate volume of about 200 to 250 cm³) along with the worker bees taken out and placed in a new hive without any gynes or queen cells in them. Then new hive was placed separately away from the parent colony, at a distance of about 5 m (Fig. 3).

Method 6: Budding method of colony division

Well-developed colonies maintained in rectangular shaped boxes were selected and a new hive box with the same shape and size was placed in front of the parent colony. A provision for the movement of foragers was created by boring a hole at the back side of the new hive, then exactly aligned with the entrance of the parent colony. Observations were made on the development of new colony in the new hive (Fig. 4). This method is similar to eduction method of division or bridging method (Mythri *et al.*, 2018) [9].

Thereafter, observations were recorded on the successful development of new queen in the divided colonies, continuation of divided colony.

The survival rate (%) of each method was calculated using the formula:

$$\text{Survival rate (\%)} = \frac{\text{No. of surviving colonies}}{\text{Total colonies divided}} \times 100$$

Results

The observation and success rate of colony division under six different artificial propagation methods

Method 1: Parallel division with presence of gynes in the divided part

Three colonies were divided using this method, out of which two colonies were successfully established while one failed. The failure was likely due to an insufficient number of worker bees remaining in the divided colony, as many workers returned to the parent colony. This is due to the divided colonies were placed close to the parent and the workers returned back to parent colony due to the attraction of the queen's pheromones. This method recorded a 66.66 per cent success rate.

Method 2: Parallel division with presence of queen cells in the divided part

Three colonies were divided using queen cells, out of which two successfully developed while one failed due to the failure of queen emergence from the queen cell in the divided part. The failure was likely caused by improper nourishment of the queen cell by the worker bees in the

divided colony. This method recorded a 66.66 per cent success rate.

Method 3: Separation of brood with presence of gynes in the divided colony

All three colonies used in this method were successfully divided and gyne placed in the divided colony developed as a new queen and new brood cells were constructed in the colony. In this method, a colony of stingless bees was successfully divided into two colonies. One of the colonies contained a old queen, while the other contained a new queen that developed from the gyne. This method recorded a 100 per cent success rate.

Method 4: Separation of brood with presence of queen cells in the divided colony

This method recorded a 66.66 per cent success rate. Three colonies were divided using queen cells, out of which two successfully developed while one failed because the queen did not emerge from the queen cell. The failure might have occurred due to stress and disturbance during the colony division process, which affected queen cell development.

Method 5: Separation of brood in absence of gynes or queen cells in the divided colony

Three colonies were divided without introducing gynes or queen cells in the divided colony. Two divided colonies were failed to develop as new colony. However in one colony emergency queen cell is formed and new queen emerged from the queen cell in the divided colony. This method recorded a 33.33 per cent success rate.

Method 6: Budding method of colony division

This method recorded a zero per cent success rate. Three colonies were divided using the budding method. Foraging activity started 2-3 days after division but no new queen and brood cells were developed in the empty/new hives of the budding method.

Discussion

Colony division is a important management practice in meliponiculture to ensure the sustainable multiplication of stingless bee colonies. Natural swarming is slow, seasonal and unpredictable which limiting the rapid expansion of colonies. Artificial division provides an efficient way to increase colony numbers, reduce dependence on wild nests

and conserve natural populations. It also helps beekeepers to meet the growing demand for pollination and honey production while maintaining genetic diversity and colony stability under managed conditions (Heard, 1999; Jailani *et al.*, 2019) ^[4, 5]. The present findings revealed that the separation of brood with gynes achieved the 100 per cent success rate followed by the separation of brood with queen cells, parallel division with gynes and parallel division with queen cells each method achieving a 66.66 per cent success rate.

The highest success was observed when the divided colonies contained either a gyne or queen cells. These results are in agreement with Kiatoko (2012) ^[6] who reported a 100 per cent success rate using separation of brood with gynes in *Meliponula ferruginea* (black) and *M. ferruginea* (reddish brown). Several studies have reported that 75-85 per cent success in colonies divided using gynes and queen cells (Mythri *et al.*, 2018; Mounika *et al.*, 2019) ^[9, 8].

Whereas, colony division without introducing gyne or queen cell in divided colony resulted lower success rate of 33.33 per cent and establishment rates. The results are similar with Gourav (2021) ^[2] who reported that, 33.33 per cent success rate using this method. Success in divided colonies obtained only during certain seasons which might be the season of development of natural queen cells or gynes in the colony and it should be active growing period of stingless bee colonies.

The colony division during honey flow period January-June, where colonies show rapid brood development (Saranya *et al.*, 2024) ^[13]. Drone production was highly seasonal, peaking between February and April, coinciding with intense brood cell construction. Mating success of new queen is closely linked to seasonal drone availability (Prato and Soares, 2013) ^[11]. The coincidence of colony division with this period of increased drone production may enhance mating opportunities for gynes, supporting the successful establishment of newly divided colonies.

No new colonies were established through budding method of division because the bees continued to utilize the parent nest and no brood development occurred in the new hive, it was likely due to the availability of sufficient space in the parent colony. Similarly, Vijayakumar *et al.* (2013) ^[15] reported that, bees accept and utilize the new hive only when there is scarcity of space for further nest expansion inside the parent colony.

Table 6: Success rate of colony division under different methods

Sl. No.	Method of division	Number of colonies		Percentage of success (%)
		Divided	succeeded	
1	Parallel division with gynes	3	2	66.66
2	Parallel division with queen cells	3	2	66.66
3	Separation of brood with gynes	3	3	100.00
4	Separation of brood with queen cells	3	2	66.66
5	Separation of brood without queen cell or gynes	3	1	33.33
6	Budding method	3	0	0.00
	Total	18	10	55.55

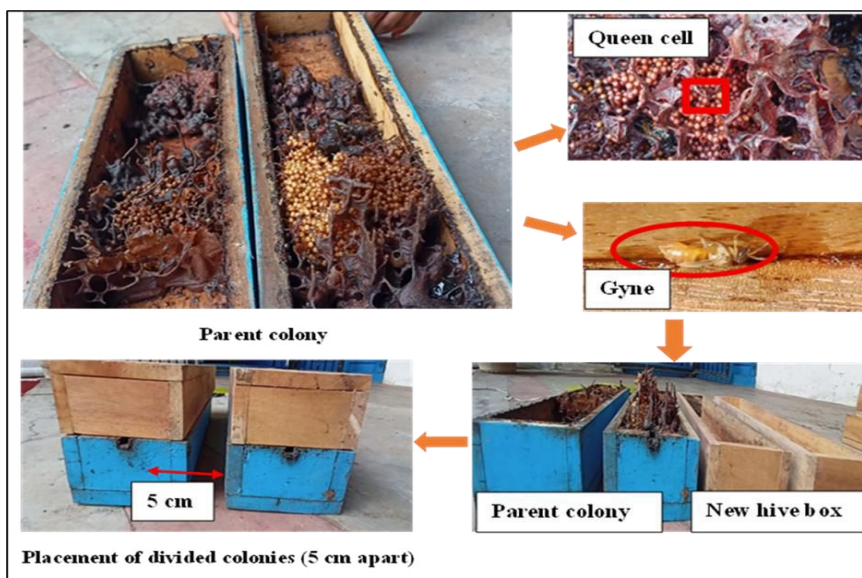


Fig 1: Parallel division with presence of gyne or queen cell in the divided part

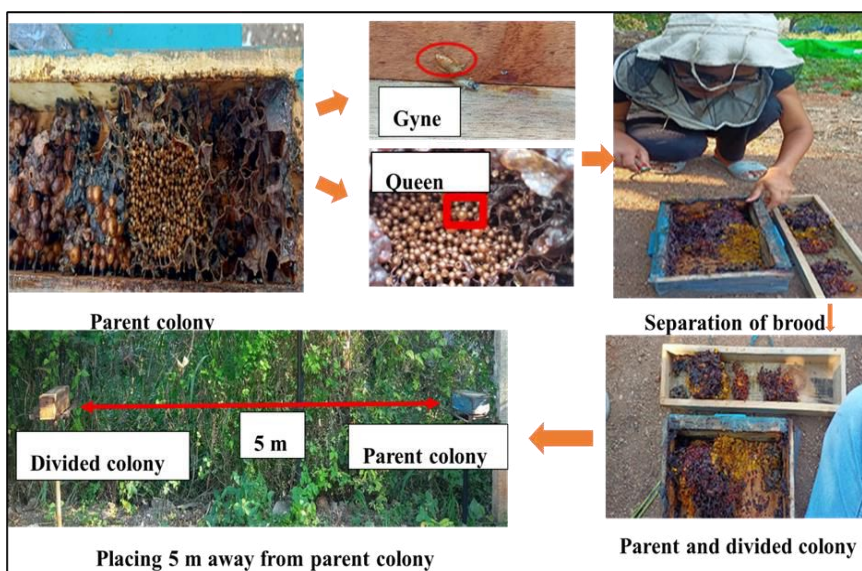


Fig 2: Separation of brood with presence of gynes or queen cells in the divided part

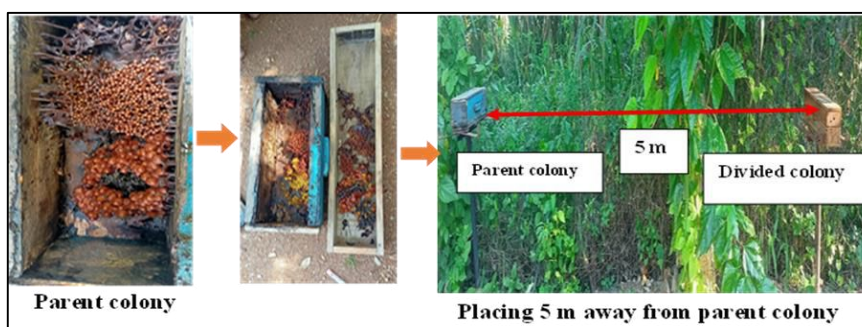


Fig 3: Separation of brood in absence of gyne or queen cells in the divided part

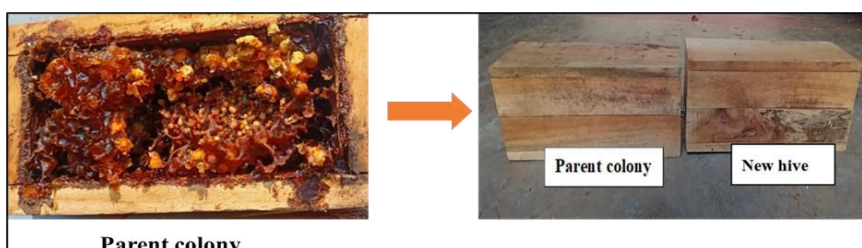


Fig 4: Budding method of colony division

Conclusion

The results of the present study revealed that the colony division in stingless bees largely depends on the presence of gynes or queen cells in the divided colonies. Whereas, absence of both gyne and queen cell resulted in much lower survival and establishment rates. Success in divided colonies obtained only during certain seasons which might be the season of development of natural queen cells or gynes in the colony.

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