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Colony propagation in stingless bees, *Tetragonula iridipennis* (Smith)

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Abstract
Suitable colony propagation techniques are lacking for *Tetragonula iridipennis* which is the most common species of stingless bees found in Tamil Nadu. Stingless bees generally produce queen cells at regular intervals. In this study, attempts were made to divide the colonies having queen cells. Eight colonies of *T. iridipennis* were divided, out of which five colonies were successfully established after the gyne emergence and egg laying of new queen. These five newly divided colonies took 40, 107, 20, 54 and 43 days for their establishment from the date of division. The failure of three divisions was due to the attack of enemies like ants, resin bees and pollen mite. The findings of this study suggested that the stingless bee colonies can be propagated easily by way of colony division with royal queen cells.

Keywords: Stingless bees, queen cell, colony division, propagation

1. Introduction
Stingless bees are one among the eusocial group of insects belonging to the family Apidae and subfamily Meliponinae. *Tetragonula iridipennis* (Smith) is the most common species found in Tamil Nadu [1]. Stingless bees differ from honey bees in certain attributes like nesting behavior, biology and provisioning of food to the developing larvae. The mechanism of caste determination and queen rearing are different in stingless bees compared to other social hymenopterans [2]. Generally in stingless bees, the gynes (virgin queen) are reared throughout the year periodically [2, 3] and the number of gynes produced depends on the species, colony population, quantity of food store available, physiological state of the physogastric queen and the reproductive cycle of the colony.
In *Trigona* genera, queens are reared in specially built royal cells provisioned with huge quantity of same quality food i.e., usually double or triple the amount of food than the worker cell illustrating the trophic method of caste determination. The newly constructed queen cells are dark brown in colour and after reaching pupation they turn into lighter colour due to scrapping of cerumen by workers and the queen varies in pigmentation ranging from pale to dark brown coloured depending upon their age [4]. The total developmental period of gyne in stingless bees takes several weeks [3] while in honey bees it is approximately 15-16 days [5]. The gynes of stingless bees cut off the top of the cell by herself leaving a circular hole while the workers and drones need assistance from older workers for their emergence [6].
Suitable colony propagation techniques are lacking for stingless bees in our country. But, the interest and awareness about meliponiculture is being increased in recent days leading to demand and scope for stingless beekeeping. So, the colonies should be multiplied rapidly to meet out the emerging demand. In *Austroplebeia*, colony propagation was practiced by removing a small portion of brood cells along with queen cell from the strong mother colony and placing it in a new hive together with food stores, young and matured workers [7].
Stingless bees construct queen cells periodically even under queenright conditions and this trait can be exploited for colony propagation. Stingless bee colonies may be divided successfully in the presence of queen cell or gyne [8]. In this study, we have attempted to propagate the colonies by the method of colony division with queen cell, pupal brood cells and pollen and resin store.

2. Materials and Methods
2.1 Study site
The study was carried out in Apiary unit, Department of Agricultural Entomology, TNAU, Coimbatore during 2018- 2019. Colony division was attempted in eight colonies of *T. iridipennis*.

2.2 Selection of colony

Strong queenright colonies with dense population of bees (approximately 2000 numbers), large brood area with queen cells and plenty of food stores such as pollen and food pot were selected for division (Fig. 1).

2.3. Preparation of hive

Rectangular wooden boxes of two litre capacity were used as hives for transferring divisioned part of colonies. The hive boxes were cleaned and resin was smeared around the entrance hole to attract the worker bees as well as get rid of entry of any intruding enemies (Fig. 2).

2.4. Division of colony

A total of eight colonies of *T. iridipennis* were subjected to colony division. Each newly divided colony was uniformly provided with 3g resin, 20 numbers of young bees (callows) and 60 adult bees from their respective mother colonies. Number of pupal brood cells and quantity of pollen (as pots) were given at varied range.

Known quantity of pollen pot (Fig.3) and worker brood cells along with a royal cell (Fig. 4) of pupal stage were separated from the mother colony and transferred to the new hive. Adult bees were collected from the mother colony in a test tube (Fig. 5) and young workers were collected using insect brush and released into new hive. A transparent polyethylene sheet was covered over the hive for easy observation and a lid was placed over it. The newly divided colony was kept in the original site and the mother colony was shifted to distant place. The divided colony was observed for new queen emergence (Fig. 6) and its establishment (Fig. 7). The date of division of colony, day of construction of pollen pot and brood cells, day of gyne emergence and first oviposition was also recorded.

2.5 Statistical analysis

Statistical analysis was done using SPSS 16.0 statistical package. Chi square test was performed to find out the association of two variables such as weight of pollen and number of pupal brood cells.

2.6 Steps involved in division of a colony

Fig 1: Strong colony

Fig 2: Resin smeared around entrance

Fig 3: Pollen pot

Fig 4: Worker and queen brood cells

Fig 5: Collection of adult workers

Fig 6: Gyne emergence from queen cell

Fig 7: Establishment of the new colony

3. Results and Discussion

The observations recorded from eight divided colonies provided with queen cell and other nest components indicated that five colonies were successfully established after the gyne emergence, mating and oviposition. The exit hole formed during emergence of the gyne was in circular shape in all the observed queen cells which was also reported in *Melipona beeechi* [6]. The newly emerged gynes observed in all the colonies were pale colored during initial days and attained tan color in subsequent days and this colouration changes in gynes was also noticed in *T. ventralis* [4].

The time taken for establishment from the date of division to oviposition was calculated for each colony and it was 40, 107, 20, 54 and 43 days respectively (Table 1). Queen cell was given at pupal stage for all the colonies except colony 2 for which queen cell was given at egg stage and it took longer period (107 days) for establishment than other colonies. The establishment of stingless bee colonies after division took more time than honey bee colonies because the gyne takes almost 50 to 70 days for its development. So the queen cells provided at pupal stage are readily accepted by the workers and also the chance of damage to queen cells while shifting at pupal stage was minimum compared to egg/larval developmental stage.

Brood cell construction was not observed in all the successful colonies before gyne emergence. The worker bees in successful colonies took about 5, 3, 6, 7 and 3 days respectively for initiating new brood cell construction from the day of gyne emergence. The new gynes took 17, 9, 11, 13 and 10 days for its first oviposition from the day of emergence and this depends mainly on physiological maturity of gynes and availability of drones for mating. The construction of new pollen and honey pots in these colonies commenced on 17, 15, 9, 9 and 8 days respectively from the date of division.

The results on the provision of pupal brood cells and pollen pot revealed that colony 1 and colony 4 were highly significant with pollen quantity of 50g and 100g respectively. Similarly the number of pupal brood cells of 215 and 50 (Table 1) was significant for colony 1 and colony 4 compared to other colonies.

In case of colony 6, damaged honey pot was also placed inside the hive while dividing, that attracted ants and further lead to the destruction of whole colony. Hence, only the pollen pot should be provided during the division of the colony. If necessary, sugar feeding as substitute can also be given after colony settlement. Gyne emergence was observed from queen cell in colony 7, but the colony was severely attacked by pollen mite subsequently leading to the death of brood and bees. In colony 8, the entrance hole was bigger which permitted the entry of resin bee and it damaged the entire colony. So these results revealed that the divided colonies should be carefully placed, monitored at regular intervals for the occurrence of pests and suitable management measures should be adopted for preventing the enemies.

Table 1: Observations on colony parameters of divided *T. iridipennis* colonies

Parameters	Colony 1	Colony 2	Colony 3	Colony 4	Colony 5	Colony 6	Colony 7	Colony 8
Date of division	17.9.18	21.12.18	10.10.18	21.11.18	10.1.19	4.10.18	4.12.18	19.3.19
No. of pupal broods given	215*	230	240	50*	150	70	300	135
Weight of pollen pot	50*	100	200	100*	100	150	150	100
Stage of queen cell	Pupa	Egg	Pupa	Pupa	Pupa	Pupa	Pupa	Pupa
No. of adult bees	60	60	60	60	60	60	60	60
No. of callows	20	20	20	20	20	20	20	20
Weight of resin	3	3	3	3	3	3	3	3
Day of construction of pollen/honey pot	3.10.18	4.1.19	18.10.18	29.11.18	17.1.19	-	6.12.18	-
Day of gyne emergence	10.10.18	18.2.19	19.10.18	1.12.18	12.2.19	-	5.1.19	-
Day of beginning of new cell construction	14.10.18	20.2.19	24.10.18	7.12.18	14.2.19	-	8.1.19	-
Day of first oviposition	26.10.18	27.3.19	29.10.18	13.1.18	21.2.19	-	-	-
Total days	40 days	107 days	20 days	54 days	43 days	-	-	-
Total days from gyne emergence to oviposition	17 days	9 days	11 days	13 days	10 days	-	-	-

Chi square value = 112.57*, * - Significance at 5% level

4. Conclusion

The results of the present study revealed that the stingless bee colonies could be propagated successfully by way of colony division with a queen cell, young and adult workers, resin, pollen pot and pupal brood cells from strong mother colonies. This method also ensures rapid development of stingless bee

colonies after gyne emergence and oviposition within shorter period of 40 to 54 days.

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