

**STUDIES ON SOME ASPECTS OF STINGLESS BEE (*Trigona iridipennis* Smith)
COLONY REPRODUCTION, CAPTURING AND MANAGEMENT TECHNIQUES**

K. PRABHU, B.Sc. (Ag.)

I. D. No. 06- 602- 009

**DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
CENTRE FOR PLANT PROTECTION STUDIES
TAMIL NADU AGRICULTURAL UNIVERSITY**

COIMBATORE – 641 003

2008

**STUDIES ON SOME ASPECTS OF STINGLESS BEE (*Trigona iridipennis* Smith)
COLONY REPRODUCTION, CAPTURING AND MANAGEMENT TECHNIQUES**

Thesis submitted in part fulfillment of the requirements for the degree

MASTER OF SCIENCE (AGRICULTURE) IN AGRICULTURAL ENTOMOLOGY

to the Tamil Nadu Agricultural University, Coimbatore

K. PRABHU, B.Sc. (Ag.)

I. D. No. 06- 602- 009

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

CENTRE FOR PLANT PROTECTION STUDIES

TAMIL NADU AGRICULTURAL UNIVERSITY

COIMBATORE – 641 003

2008

CERTIFICATE

This is to certify that the thesis entitled “**Studies on some aspects of stingless bee (*Trigona iridipennis* Smith) colony reproduction, capturing and management techniques**” submitted in part fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) IN AGRICULTURAL ENTOMOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a record of bonafide research work carried out by **Mr. K.PRABHU** under my supervision and guidance and that no part of the thesis has been submitted for the award of any degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

Place: Coimbatore

Dr. M. MUTHURAMAN

Date :

Chairman

Approved by

Chairman: **(Dr. M. MUTHURAMAN)**

Members: **(Dr. P.KARUPPUCHAMY)**

(Dr. K. SOORIANATHASUNDARAM)

(Dr. K.VENKATESAN)

Date:

(EXTERNAL EXAMINER)

CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
I	INTRODUCTION	1
II	REVIEW OF LITERATURE	4
III	MATERIALS AND METHODS	14
IV	RESULTS	27
V	DISCUSSION	68
VI	SUMMARY	99
	REFERENCES	103

LIST OF TABLES

Table No.	Title	Page No.
1.	Basic data on feral colonies found in a nest congregation site	17
2.	Nest entrance architecture of colonies captured from wall cavities	28
3.	Distribution of resin dumps	29
4.	Relative distribution of food pots	32
5.	Distribution and attachment of brood clusters	33
6.	Nest associated drones activity in nest congregation sites	35
7.	Activity of drones at non-nest associated drone congregation sites	38
8.	Variations in colony number at nest congregation area	40
9.	Sequence of normal swarming	41
10.	Colony founding by normal aggressive swarming	42
11.	Establishment and loss of colonies due to sequential aggressive swarming	46
12.	Emergency queen rearing	48
13.	Site of occurrence of queen inside the nest	51

Table No.	Title	Page No.
14.	Requeening of orphan colonies with queen cells	53
15.	Requeening of orphan colonies with gynes of unknown age	55
16.	Requeening of orphan colonies with gynes of known age	56
17.	Requeening orphan colonies with laying queens	57
18.	Time of commencement and time of cessation of bee visitation in aonla	59
19.	Foraging time required for resin foraging from plant sources	67

LIST OF FIGURES

Figure No.	Title	Page No.
1.	Floral handling time of <i>T. iridipennis</i> on different aonla varieties	60
2.	Effect of extent of flower opening on floral handling time of <i>T. iridipennis</i> in aonla variety BSR-1	60
3.	Inflorescence handling time of <i>T. iridipennis</i> on different aonla varieties	61
4.	Floral visitation rate of <i>T. iridipennis</i> on different aonla varieties	61
5.	Inflorescence visitation rate of <i>T. iridipennis</i> on different aonla varieties	62
6.	Pollen foraging time and inflorescence visitation rate for fully loading corbiculae	62
7.	Peak foraging activity of <i>T. iridipennis</i> on different aonla varieties	65
8.	Origin and fate of normal and aggressive swarms in stingless bee colonies	78
9.	Management of aggressive swarms	79
10.	How to save an orphan colony?	93

LIST OF PLATES

Plate No.	Title	Page No.
1.	Non-nest associated drone congregation	15
2.	Dilapidated building housing feral colonies	20
3.	Queen cells	20
4.	Gyne	21
5.	Laying queen	21
6.	Aonla nursery	24
7.	View of an exposed nest	31
8.	Adjacently placed food pots	31
9.	Distribution of brood clusters	34
10.	Attachment of brood clusters	34
11.	Nest associated drone congregation	36
12.	Guard bees at the entrance	44
13.	After effects of aggressive swarming	44
14.	Mobbing flight of disturbed bees	47
15.	Emergency queen cells	47
16.	Queen cell linked to an auxiliary cell	49
17.	Worker cell linked to an auxiliary cell	49
18.	Sings of queenlessness	52
19.	Pollen foraging from aonla flowers	63
20.	Resin collection from injured tree bark of mango	68
21.	Resin flowing out from jack fruit stalk	68

Plate No.	Title	Page No.
22.	Drones resting in a group	73
23.	Drones resting on a twig	73
24.	An aggressive swarm invading a box hive	77
25.	Pricking method	85
26.	Hammer tapping for nest location	85
27.	Queen lifting by using a fork	88
28.	Captured queen inside a plastic container	88
29.	Resin application around the hive entrance	89
30.	Refixed entrance tube	89
31.	Safe removal of flightless bees	91
32.	Hive partitioning with dummy board	91
33.	Inflorescence of aonla	95
34.	Foragers trapped in resin	97
35.	Intraspecific resin robbing	97

ACKNOWLEDGEMENT

*It is a life time opportunity to work under the guidance of **Dr.M. Muthuraman**, Professor of Agrl. Entomology and Chairman of the advisory committee. I express my deep sense of gratitude to my chairman. His motivation, unstinted encouragement, affection and care were the great sources of inspiration which enabled me to successfully complete this fascinating research work.*

*I feel immense pleasure in extending my special thanks to the members of advisory committee, **Dr.P. Karuppuchamy**, Professor (Agricultural Entomology), **Dr.K.Soorianathasundaram**, Professor (Horticulture) and **Dr.K.Venkatesan**, Associate professor (Horticulture) for their constructive criticism, scholarly advice, constant encouragement and timely help rendered during the course of this study.*

*I record my deep sense of gratitude to **Dr.P. Sivasubramaniam**, Professor and Head, Department of Agricultural Entomology and **Dr.S. Kuttalam**, Professor (Agricultural Entomology), for their help extended during the study period.*

*My special thanks are due to **Dr.P. Veerabhadhiran** Professor (Plant Breeding and Genetics), **Dr.P.K.Selvaraj**, Professor (Soil and Water conservation), **Dr.P.M.Shanmugam**, Assistant Professor (Agronomy), **Dr.S.Natarajan** Assistant Professor (Agronomy), **Dr.M.Vijayakumar**, Assistant Professor (Agronomy), and **Dr.E.Rajeshwari**, Assistant Professor (Plant Pathology) who have been kind enough in giving a helping hand at various stages of my research work. I am grateful to **Dr.M. Balusamy**, Professor and Head, Agricultural Research Station, Bhavanisagar for providing all help needed for carrying out my research at Bhavanisagar.*

*I am indebted to **Mr. Sajhan Jose**, Lecturer, St. Joseps, College, Moolamattam, Kerala for sparing some important reprints on emergency queen rearing.*

*I thank **Dr.S. Vijayaragavan**, Retired professor of Entomology for the help rendered in translating a research article in German to English*

*The field help provided by **Mr.K. Udayakumar** in capturing feral colonies will be ever remembered. I thank **Mr.G.K.Thangavelu**, Bee keeper, Ganapathy and **Mr. Pandiarajan**, Sular for their photographic and video documentations.*

*I express my profound thanks to my best friends **Mr. R. Nagganatha Suganthan, S. Srinivasan, A. Balusamy, T. Chandrasekar, C. Krishnamoorthy and B. Venudevan** for their affection and guidance offered to me throughout my research.*

*I express my sincere thanks to my beloved seniors **Mr. S. Ranjith Kumar**, and **Mr.Govindan**, and my classmates **Gladis, Jadhav, Jayaprakash, Kennedy, Karthik, Kavitha, Murali, Natesan, Raja, Rajadurai, Ramesh, K.Sridhar, N.Sridhar Swaminathan, Sudha, Thanavendan**, and **Vijay** for their help and support.*

I will ever remember with gratitude the love, affection and financial support extended by my parents during the course of this study. I thank every one who helped me in some way or other for successfully completing my research.

K. PRABHU

ABSTRACT

STUDIES ON SOME ASPECTS OF STINGLESS BEE (*Trigona iridipennis* Smith) COLONY REPRODUCTION, CAPTURING AND MANAGEMENT TECHNIQUES

BY

K. Prabhu

Degree : **Master of Science in Agricultural Entomology**

Chairman : **Dr. M. Muthuraman**

Professor (Agrl. Entomology)

Plant Clinic Centre

Agricultural Research Station

Bhavanisagar- 638 451

2008

Stingless bees (*Trigona iridipennis*) often built nests in wall cavities. Several such nests were found together forming a nest congregation. The number of colonies in a nest congregation often fluctuated due to the entry of new swarms and exit of some existing colonies. The nest entrance architecture and the arrangement of nest components varied according to the nature of the wall surface and size of the wall cavity. Drones formed both nest associated and non-nest associated congregations in proximity to nest congregation sites. Drone swarms were usually found active in front of nests having gynes for varying periods. Drones also collected nectar

from flowers during day time and formed resting clusters on twigs and leaves during night time. Colony reproduction in stingless bees occurred through two types of swarming *viz.*, normal swarming and aggressive swarming. The normal swarming sequence leading to the birth of a new daughter colony was found out. Swarms were attracted to both old deserted nests and new nesting spaces available in nest congregation sites and an empty box hive once occupied by a colony. However, aggressive swarms established new colonies by killing and evicting an already established colony either through single invasion or multiple invasions. Severe intraspecific competition for nesting space also paved way for aggressions which resulted in the death of colonies and hampered the colony reproduction. Queen rearing under emergency impulse created by dequeening was discovered for the first time in *T. iridipennis*. A worker larva was turned into a queen by provisioning with additional quantity of brood food. Simple methods were found out to hive feral colonies from wall cavities safely and successfully. Simple techniques were designed for safe removal of entrance tube, stones, food pots, brood nest, queen and flightless young bees at the time of hiving. Fixing the entrance tube externally to the hive entrance and smearing resin internally around the entrance hole resulted in quick settling of evicted bees in the box hive. Appearance of fake queen cells in a newly hived colony was a sure sign of queenlessness. Queenless colonies readily accepted introduced ripe queen cells, newly emerged gynes and laying queens. Stingless bees were the dominant pollen foragers on aonla and their intense pollen collection activity was found from 8.00 to 9.00 a.m. and 5.00 to 6.00 p.m. during the peak flowering period (February-March) at Bhavanisagar conditions. They also actively collected resin from jack fruit, mango bark and trunk of peepul tree.

ABSTRACT

STUDIES ON SOME ASPECTS OF STINGLESS BEE (*Trigona iridipennis* Smith) COLONY REPRODUCTION, CAPTURING AND MANAGEMENT TECHNIQUES

BY

K. Prabhu

Degree : **Master of Science in Agricultural Entomology**

Chairman : **Dr. M. Muthuraman**

Professor (Agrl. Entomology)

Plant Clinic Centre

Agricultural Research Station

Bhavanisagar- 638 451

2008

Stingless bees (*Trigona iridipennis*) often built nests in wall cavities. Several such nests were found together forming a nest congregation. The number of colonies in a nest congregation often fluctuated due to the entry of new swarms and exit of some existing colonies. The nest entrance architecture and the arrangement of nest components varied according to the nature of the wall surface and size of the wall cavity. Drones formed both nest associated and non-nest associated congregations in proximity to nest congregation sites. Drone swarms were usually

found active in front of nests having gynes for varying periods. Drones also collected nectar from flowers during day time and formed resting clusters on twigs and leaves during night time. Colony reproduction in stingless bees occurred through two types of swarming *viz.*, normal swarming and aggressive swarming. The normal swarming sequence leading to the birth of a new daughter colony was found out. Swarms were attracted to both old deserted nests and new nesting spaces available in nest congregation sites and an empty box hive once occupied by a colony. However, aggressive swarms established new colonies by killing and evicting an already established colony either through single invasion or multiple invasions. Severe intraspecific competition for nesting space also paved way for aggressions which resulted in the death of colonies and hampered the colony reproduction. Queen rearing under emergency impulse created by dequeening was discovered for the first time in *T. iridipennis*. A worker larva was turned into a queen by provisioning with additional quantity of brood food. Simple methods were found out to hive feral colonies from wall cavities safely and successfully. Simple techniques were designed for safe removal of entrance tube, stones, food pots, brood nest, queen and flightless young bees at the time of hiving. Fixing the entrance tube externally to the hive entrance and smearing resin internally around the entrance hole resulted in quick settling of evicted bees in the box hive. Appearance of fake queen cells in a newly hived colony was a sure sign of queenlessness. Queenless colonies readily accepted introduced ripe queen cells, newly emerged gynes and laying queens. Stingless bees were the dominant pollen foragers on aonla and their intense pollen collection activity was found from 8.00 to 9.00 a.m. and 5.00 to 6.00 p.m. during the peak flowering period (February-March) at Bhavanisagar conditions. They also actively collected resin from jack fruit, mango bark and trunk of peepul tree.

CHAPTER I

INTRODUCTION

Stingless bees are social bees like the stinging honey bees. In contrast to *Apis*, these bees mate only one time, do not use pure wax to build their nest, do not use water to cool it, can not freely swarm to reproduce and drones feed on flowers, while the gravid queen can not fly. They live in tropical and subtropical areas. They have reduced wing venation. Though they are stingless, they protect their nest very effectively by biting the intruders with their mandibles supported by strong muscles. Defense behaviour of stingless bees is much less hurtful to the bee keepers than honey bees.

Stingless bees have populated 65 million years longer than *Apis*. They have 50 times more species which are both distinctive and diverse. There are over 600 species of stingless bees grouped under 56 genera. They belong to the family Apidae and are classified under the sub family Meliponinae. *Melipona* and *Trigona* are the two important genera classified under Meliponinae. *Melipona* is restricted to tropical America while *Trigona* is the largest group and most widely distributed and occurs from Southern Asia to Australia (Crane, 1992). The most common stingless bee species of South India is *Trigona iridipennis* Smith. This species was earlier described as *Melipona iridipennis* by Smith. However, it is now accepted that the species *iridipennis* belongs to genus *Trigona* and not *Melipona*. *T. iridipennis* was redefined as a species belonging to India and Srilanka (Michener, 1974). These species are black with white hairs on face and sides, iridescent wings and triangular abdomen.

The art of keeping stingless bees is known as meliponiculture. This activity is generally taken up by traditional communities. The challenges, opportunities and global status of meliponiculture have been reviewed recently (Cortopassi-Laurino *et al.*, 2006). Stingless bee beekeeping is traditionally practiced in Brazil, México and Costa Rica using log hives and box hives. Many macadamia growers in Australia are also purchasing their own stingless bee

colonies for pollination. The main species used for pollination are *T.carbonaria* and *T.hockingsi* (Heard, 1994). *Trigona* species are mass produced in Philippines for pollination and honey production in halved coconut shells (Facade and Cervancia, 2003).

Honey, resin and pollen are important hive products one can obtain by keeping stingless bees. In general stingless bee colonies make far less honey compared to honey bees. Stingless bee honey is used much more as a medicine than as a sweetener. The *Trigona* honey carries higher price than the honey of *Apis*. In addition, the stingless bee colonies can be used for planned pollination (Heard, 1999). Hence, a stingless bee beekeeper can earn money by renting of colonies for pollination service. The use of stingless bees as crop pollinators has opened a new economic possibility for meliponiculture. *Trigona spp.* play a crucial role in effecting pollination in aonla, mango, physic nut, coconut and chow chow in India .

Research on stingless bees was initiated in Tamil Nadu in 2000 by Swaminathan. He fixed the correct identity of the most common species occurring in Tamil Nadu as *T.iridipennis*. He also documented the pasturage sources and nesting biology of stingless bees. Subsequently the genetic variation among different population of *T.iridipennis* was first documented through DNA analysis (Sriram, 2004). The provisioning and oviposition process in this species was studied in detail and developmental period of workers was found out. In addition, both intranidal and extranidal behaviour of drones and replacement of aged queen by gyne were found out. (Saravanan, 2005)

Much research on nesting biology has been done on neotropical stingless bees because roughly three fourth of all species are American (Camargo and Pedro, 1992). Similarly much work on colony reproduction has been done pertaining to *Melipona* (Veen van, 1999). However, only limited research work has been carried out so far on developmental biology and colony reproduction in *T.iridipennis*. Likewise research studies on the foraging behaviour of these bees on fruit crops and resin collection behaviour from resin flora are limited. Lack of availability of

colonies and standardized meliponiculture techniques are the two major constraints now faced in popularizing meliponiculture among farmers and bee keepers. One can start meliponiculture by hiving feral colonies from their natural habitat (Muthuraman and Thriugnanasambantam, 2003). The hiving techniques developed by these authors are yet to be refined and standardized. One possible way to obtain colonies for establishing a meliponary is to capture natural swarms by offering suitable artificial nesting sites. A thorough understanding of reproductive biology of stingless bees is essential for proper colony multiplication and development of husbandary techniques. The process of swarming has not been studied in *T. iridipennis*. The method of emergency queen cell production in stingless bee colonies is totally different from honey bees. Such emergency queen replacements have been reported in *T. varia* (Faustino *et al.*, 2002) but not reported in *T. iridipennis* so far. To fill some of the above research gaps existing in stingless bee biology and meliponiculture, the present study was taken up with the following objectives.

- 1) to study the nesting biology of stingless bees in wall cavities.
- 2) to investigate the behaviour of drones.
- 3) to understand the swarming behaviour and emergency queen rearing
- 4) to standardize the methods of capturing feral colonies.
- 5) to evolve techniques for saving orphan colonies.
- 6) to study the methods of pollen foraging in aonla and resin foraging from selected trees.

CHAPTER II

REVIEW OF LITERATURE

The literature relevant to the research topic is reviewed here under.

2.1. Nest architecture

The nesting biology of stingless bees has been recently reviewed by Roubik (2006). Stingless bees are cavity nesting bees. The nest is the central place from which stingless bees mate, forage and pass through their life stages. Tree cavities are the most common nesting sites of stingless bees. They usually build their nests inside a hollow branch or dead tree trunk. Apart from tree cavities they also construct their nests inside wall cavities. Both mud walls and stone walls offer excellent nesting sites for these bees.

Availability of numerous nesting sites inside a wall often results in the occurrence of nests side by side leading to the formation of nest congregation. Aggregation of colonies occurs on natural and artificial substrates including building or hut walls and roofs. As many as 84 colonies of *T. fuscobalteata* and *T. sapiens* were observed in a bamboo farm house inside bamboo stem cavities in the Philippines by Starr and Sakagami (1987). Likewise 16 colonies were found inside a mud wall at one location (Swaminathan, 2000) and 111 colonies were found inside a dilapidated building at Sirumugai by Sriram (2004).

2.1.1. Nest entrance architecture

A conspicuous entrance tube is found in front of the nest. It is a passage way connecting the colony with outside. Normally each nest has one entrance tube of varying length made of cerumen. It is usually sticky in a live nest and becomes brittle in a deserted colony. It is soft and pliable when newly built. Later it turns darker due to maturation. In general the width of the entrance opening is related to the number of bees in the colony. Nests with wider entrance have

more number of guard bees and allow more bees to enter and exit simultaneously. Nest openings are big enough to avoid traffic jams and small enough so that defense is still possible (Biesmeijer, 1999). The entrance tube architecture is a species specific character. The entrance tube is slender and sticky in *T. moorei* (Sakagami *et al.*, 1989). The external entrance tubes of *T. gribodoi* are 6 to 25 mm long and project at an angle to the bark (Pooley and Michener, 1969).

Bees obviously need an entrance to enter and leave the nest. They collect the food and building material into the nest and throw out the waste material produced inside the colony through this tube. The main purpose of the entrance tube is to keep off both rain and natural enemies from entering into the nest. They deposit fresh resin on the external entrance tube which may deter and act as a barrier against ants. However, the chemistry and choice of nest resins useful as repellents for the natural enemies of bees are yet to be studied. The entrance tube is also useful in nest navigation.

The bees often build a passage way or internal tunnel leading from entrance to the inner parts of the nest. A few resin dumps are found always nearer to internal tunnel either on nest floor or wall. The guard bees use resin as a sticky weapon to fight off intruders. They also use the resin as a building material for nest construction along with wax.

2.1.2. Food pots

Normally there will be single food storage zone. But in some nests found in stone wall cavities the storage zone and brood zone are found one after another. Food pots are small to large spheres or egg-shaped or even conical or cylindrical. Often these food pots are pressed together in odd conglomerates. Mostly in many nests the storage zone is divided into a pollen area and honey area. In natural colonies honey and pollen are stored in separate pots. Sometimes these pots are often intermixed. Pollen pots are closer to the entrance. Honey pots are often constructed in the outer parts of the nest with soft cerumen which hardens subsequently. Pollen

pots are tougher than honey pots. Depending on spatial limitations, food pots are built either above or below or at both sides of the brood nest one over the other or side by side. Often a cluster of food pots will contain both honey pots and pollen pots (Dollin, 1996). Pollen pots are sealed when they are full and honey pots are sealed when the honey is ripened. Honey pots are built of cerumen, a mixture of wax and plant resin. In general colonies make far less honey and store it in thin walled honey pots. They store surplus honey available during honey flow season in nest cavity extremes. The incorporation of plant resins adds organic substances that are believed to possess and impart antibiotic properties to the stored honey (Gilliam *et al.*, 1985).

2.1.3. Nest envelopes

The larger cavities are often limited by special plates called batumen which are usually made of hard cerumen. The walls of the nest cavity are frequently lined with a thin layer of cerumen called lining batumen (Michener, 1946). The batumen gives protection against possible enemies and also serves as a protection against water (Velthuis, 1997). A single or multiple layers of soft cerumen called involucreum may surround and protect the brood nest. Very rarely an involucreum is seen inside the nest of *T. iridipennis*

2.1.4. Brood nest

The brood nest is a very dynamic structure which changes in shape continuously due to continuous turn over of combs (De Bruijn, 1994). The brood cells are smaller, spherical to ovoid shaped, open upwards and cluster together in a group. Cluster type of brood nest enables *T. iridipennis* to build nests even in narrow and irregularly shaped cavities. Clustered brood cells are arranged in connected chains (Michener, 2001). Cerumen pillars are used as anchors for attaching the brood nest inside the nest cavity.

Brood production in stingless bees is similar to that of solitary bees. Brood cells house eggs and larvae. The larval brood cells are built of brown cerumen. Prior to pupation the larva

spins a silken cocoon. Much of the brood cell cerumen is stripped away leaving the cocoons largely exposed. Thus the cerumen coat is immediately removed from a newly spun pupal cocoon and reutilized for brood cell construction. Queens are reared in large cells built generally on margin. These cells are large and found on the edge of the comb. In *Trigona spp.* the worker cells and drone cells are similar (De Bruijn, 1994).

2.1.5. Waste dump

Waste dump is another nest component commonly found in stingless bee nests. These dumps function as latrines where workers defecate and deposit the remains of cocoons and dead bees. Several nest symbionts also live in waste dumps. (De Bruijn, 1993).

2.2. Drone congregation

Drones are produced only from queen laid haploid eggs in *T. iridipennis* since reproduction by workers is totally absent in this species. Drones are produced throughout the year in this species (Swaminathan, 2000). Chinh *et al.* (2003) have found out that male production occurs throughout the year at population level and in clumped periods at colony level. They found that drone production is positively correlated with number of brood cells, food pots and emerging workers.

Drones apparently do not perform any task inside the nest that benefit the colony. Drones are usually fed by workers. They also take honey from honey pots occasionally. Stingless bee drones are capable of taking food also from flowers. This foraging ability allows them to survive for several days outside the hive. Drones leave the hive when they acquire flying ability. They do not return to their parental nest after their departure. Nest associated drone congregations are common in all the species of *Trigona* since they are weak fliers. They improve their chances of mating by forming congregations near the nests. Occasionally drones leave the congregation site

for sometime probably for foraging. They are found to participate in more than one congregation. They perform short group flights in front of the entrance in which virgin queens are present. They hover around the nest entrance from dawn to dusk with intermittent short periods of rest. In *T. iridipennis* after dusk they took rest in groups on nearby plants and twigs (Saravanan, 2005).

Such drone swarms were found in front of the nest in *M. favosa* between 7.00 a.m. to 6.00 p.m. and showed major activity and density late in the morning and in afternoon that paved way for out breeding (Sommeijer and De Bruijn, 1995). The period of occurrence of drone congregation varied from place to place but it always coincided with the period peak production of sexuals which alone resulted in successful mating and perpetuation of newly formed colonies and increased the rate of colony survival. Drones of *M. beechii* collected food from flowers for sustaining their life and were capable of living independently of the natal nest (Veen van *et al.*, 1997). Rain negatively influenced the flying activity of drones in *Tetragonisca angustula* (Veen van and Sommeijer, 2000).

2.3. Colony reproduction

2.3.1. Swarming

Colony reproduction commonly occurs in honey bees and stingless bees through swarming. Swarming is a natural process that gives birth to new bee colonies which leads to colony multiplication. In contrast to *Apis* the stingless bees colonies reproduce only once a year or even less frequently (Bego, 1982; Roubik, 1989). The sequence of swarming process in stingless bees was first described by Nogueira-Neto (1954). It is interestingly different from that of honey bees (Michener, 1974; Sakagami, 1982). In stingless bees the following steps are described. Scout bees look for a suitable nesting site closer to the mother nest. The selected site is cleaned. The swarming occurs only after making a new domicile. Workers transport cerumen and propolis from mother nest.

The quantum of building materials transported depends on the state of the cavity and crevices found inside it. Food pots are built and provisioned with pollen and honey. The process of building food pots and provisioning goes on for weeks. They carry building materials in their corbiculae and food in their crop. The virgin queen departs with a mass of workers to the newly constructed nest. Nest associated drone congregation occurs in front of new nests occupied by swarms. The young gyne makes a mating flight from the new nest and returns mated with a mating plug. Unlike in *Apis* the gyne mates once with a drone. The mated queen starts laying eggs in brood cells provisioned with brood food. The brood cells are closed immediately after egg laying (Nogueira-Neto, 1954; Silva da *et al.*, 1972; Michener, 1974; Wille and Orozco, 1975; Sakagami, 1982; Engels and Imperatriz-Fonseca, 1990).

The detailed information on swarming process is available for *T. laeviceps* for which six swarm processes are described (Inoue *et al.*, 1984). The size of the worker population and ample pollen stores are found to be essential for colony reproduction. The authors found for the first time that about half of the workers that had entered the daughter nest with the swarm returned to the mother colony the next day, a behaviour which probably helped to adjust the number of workers in the daughter nest and mother nest.

The conditions of the mother nest, the investment in resources and workers in the daughter colony, the influence of environmental factors, availability of suitable nesting sites and predation were found to influence the swarming behaviour (Engels and Imperatriz-Fonseca, 1990; Inoue *et al.*, 1984; Roubik, 1989). Introduction of artificial nesting sites in orchards increased the colony density of *T. minangkabau* by two to five times (Inoue *et al.*, 1993). In Southern Brazil some bee keepers use empty bottles to attract stingless bee swarms of *Tetragonisca angustula* (Ratnieks and Hart, 2001)

Colony spacing is accomplished in nest congregation areas by aggressive prevention of new colony establishment. The elements of mechanism of colony spacing are suggested to be

mediated by pheromones. Pheromones are useful for marking of potential nest sites, recruitment of workers and aggression between workers from rival nests. (Hubbell and Johnson, 1977). Swarms may originate from near by nests at the same time in *T. carbonaria*. In such situations conflicts may arise between bees of different swarms and may attack one another and wrestle to death. Some times a strong colony may issue more than one swarm, so two separate groups could be established which fight it out in the swarms (Dollin, 2002)

2.3.2. Emergency queen rearing

The reproductive strategies of *Melipona* and *Trigona* are different. In Trigonine bees workers build large brood cells called royal cells in which queens are reared as in honey bees. However, there is no difference in the quality of brood food provided to the larvae of queens and workers. But the quantum of food given to the queen larva is more which results in caste differentiation. Queen plays a vital role in the survival of colony. The colony population depends on the egg laying capacity of the queen. Supersedure takes place when the productivity of queen diminishes. In stingless bees gynes are produced throughout the year (Michener, 1974; Sakagami 1982; Veen van *et al.*, 1992). It is hypothesized that the reproductive competition between the physogastric queen and the permanently present gynes is a mechanism by which the quality of laying queen is continuously tested (Koedam *et al.*, 1995)

The presence of more than one gyne in a populous colony helps in queen replacement. In stingless bees caste differentiation can not be influenced once a brood cell has been sealed after ovipositions because of the larvae are mass provisioned. Therefore in stingless bees queen replacement after the loss of a queen depends either on the presence of sealed queen cells or already emerged virgin queens. When such possibilities do not exist the colony becomes hopelessly queenless. In such colonies there will be a gradual decline in worker population resulting in colony death.

As in honey bees in Trigonine bees queen rearing occurs under three conditions *viz.*, swarming, supersedure and emergency impulse. The queens reared under emergency impulse are provided with extra larval food kept in an auxiliary cell which is attached to the worker brood cell via a feeding connection. Such type of requeening process has been described in some species of Trigonine bees (Faustino *et al.*, 2002). Earlier it was believed that emergency queen rearing is impossible in stingless bees because of mass provisioning system of larval feeding (Sakagami, 1982). However, now it has been proved that Trigonine stingless bees can also rear queens whenever queens are lost.

2.4. Hiving feral colonies

Meliponiculture can be popularized only when stingless bee colonies are made available to those who are interested in stingless bee keeping. Hiving feral colonies is an important option available for acquiring stingless bee colonies. The method of hiving stingless bee colonies (*T. iridipennis*) both from wall cavities and tree cavities was reported by (Muthuraman and Thirugnanasambantam, 2003). They have reported some important techniques for exposing the feral nests, removing nest components and transferring of queen and workers. They have indicated the potential of resin and entrance tube in attracting the bees from feral nest to box hives. In some parts of Kerala feral colonies *T. iridipennis* are transferred either into bamboo nodes or mud pots or coconut shells and kept in backyard mainly for honey production. Similarly halved coconut shells are used for hiving stingless bees in the Philippines. Such hiving procedures have been developed in Australia for transferring natural colonies of *T. carbonaria* found inside tree cavity into box hives (Heard, 1988). Stingless bee colonies can be reared without transferring or relocating the existing nest from wild. This method can be used to harvest honey and pollen from colonies established in walls and elsewhere (Fajardo and Cervancia, 2003).

Of late methods have been standardized for multiplying stingless bee colonies through colony division. Two types of nest splitting *viz.*, vertical splitting and horizontal splitting are advocated for multiplying the colonies (Dollin and Heard, 1997; Saravanan, 2005). New daughter colonies can be produced by dividing the brood nest of a strong colony containing queen cells. The split with queen is kept in the new place and the queenless split kept in the same place to allow it to rear its own queen. The optimum brood size for artificial propagation of stingless bee, *Scaptotrigona mexicana* has been found to be 50 g of pupal brood having approximately 2750 mature pupae (Arzaluz Gutierrez *et al.*, 2002).

2.5. Requeening of orphan colonies

Orphan colonies of honey bees are often requeened either by using ripe queen cells or mated queens. Several queen introduction methods are followed to make the orphan colonies to accept the alien queen (Mc Cutcheon, 2001). Such queen introduction studies are wanting for stingless bees.

2.6. Foraging behaviour

Stingless bees are generalist flower visitors. They visit a broad range of plant species. *T. iridipennis* visited 60 plants for pollen and nectar (Swaminathan, 2000). They prefer small flowers, dense inflorescences and flowers with long corolla tubes that are wide enough for the bees to enter.

Stingless bees visited both pistillate and staminate flowers of coconut. The pollen foragers after visiting staminate flowers also visited the pistillate flowers of coconut there by effecting efficient pollen transfer (Mc Gregor, 1976). They were the most common insect visitors of mango inflorescence in India (Singh, 1989). In addition to flowers, *T. iridipennis* also collected fruit juice, and honey dew excreted by aphids for honey making (Swaminathan, 2000). Like honey bees the stingless bee, *T. carbonaria* showed flower constancy which was found out

by analyzing the composition of pollen loads of the individuals over a period (White *et al.*, 2001). Both honey bees (*A. florea*, *A. cerana indica*) and stingless bee (*Trigona spp.*) were the important floral visitors in aonla (Anonymous, 2002). The stingless bee, *T.collina* unusually collected the spores of the mould, *Rhizopus sp.* in lieu of pollen (Eltz *et al.*, 2002). Stingless bees were found to be the main pollinators of mango, aonla, bael, litchi, guava and jamun (Shashi Sharma, *et al.*, 2004). A recent study has indicated the utility of physic nut, a biofuel crop (*Jatropha curcas*) as valuable bee pasturage both for honey bees and stingless bees (Saravanan, 2005).

In addition to pollen and nectar the workers of stingless bees also collect water and resin. Water is mainly collected for diluting honey. Since they are found of collecting resin they are also called resin bees or dammer bees. Resin is used by stingless bees for various purposes. Stingless bees require a large quantity of resin for nest building. *T. iridipennis* was found to collect resin or latex from eleven plants (Swaminathan, 2000). These bees also collect latex from *Synadenium grantii*, resin from cut surfaces of branches and tree trunks and cut surfaces of jack and fig by resorting to group foraging strategy (Saravanan *et al.*, 2004).

CHAPTER III

MATERIALS AND METHODS

Basic studies were taken up to find out certain aspects of colony reproduction and foraging behaviour of the commonly occurring stingless bees, *Trigona iridipennis*. Applied research was also carried out to develop standard techniques for hiving feral colonies of stingless bees from wall cavities and requeening of queenless colonies. The materials used and the methodologies followed in the above research studies are presented in this chapter.

3.1. Nest architecture

Nesting biology of *T. iridipennis* nesting in stone wall cavities was studied. Metric data pertaining to nest entrance architecture, number of resin dumps, relative distribution of food pots and brood clusters were recorded in 40 feral colonies

3.2. Drone congregations

Behaviour of stingless bee drones outside their nest was studied over a period of six months from October 2007 to March 2008. Both nest associated and non-nest associated drone activity were documented during the study period. Nest associated drone activity was studied both at the nest congregation site and in the meliponary. The status of the colonies that triggered the hovering of drones in front of the nest or hive was observed and recorded. In addition, the total number of days spent by drones for such activity and daily data relating to time of commencement, time of cessation and duration of such drone flight behaviour were recorded. Such data were also taken for non-nest associated drone congregations on neem and jack tree trunks and near a bamboo pole projecting from the roof of a dilapidated building housing several feral colonies of stingless bees (Plate 1).

3.3. Colony reproduction

3.3.1. Swarming

3.3.1.1. Normal swarming

Swarming sequence in stingless bees was studied at two locations. Trap hives were set up at two different nest congregation areas in Bhavanisagar. Both fresh split bamboo node hives of 40 cm length and 8 cm diameter and well-cleaned old hives (22 x 12.5 x 14.5 cm) were used to trap the swarms. Five new empty hives *i.e.*, three bamboo node hives and two box hives were kept at the nest congregation site for trapping the swarms. Resin residue obtained from honey pots after honey extraction was melted in a hot water bath. The molten resin was smeared inside the trap hives using a brush. Trap hives were hung from the rooftop of two old buildings housing nearly hundred feral colonies of stingless bees. The trap hives were periodically inspected at weekly interval for a period of eight months from September - 2007 to April - 2008 to ascertain the settling of swarms. The natural swarming sequence was studied by using a swarm which settled inside a box hive (22 x 12.5 x 14.5 cm) once occupied and deserted by a stingless bee colony containing various nest components. Observations were taken periodically commencing from nest selection by scout bees until the initiation of egg laying by mated gyne.

The swarming behaviour of stingless bee was studied in a nest congregation site at Bhavanisagar. At the time of initiation of this study there were 67 feral colonies inside the walls of an old building. Basic data about the nest congregation used for swarming studies are given in Table (1-A to C). The change in number of colonies at the study site was monitored over a period of eight months from August to March 2008. Monthwise data pertaining to arrivals and establishment of new swarms and desertion of already existing colonies were collected.

3.3.1.2. Aggressive swarming

Colony founding by aggressive swarms was studied at the same nest congregation site chosen for swarm trapping technique. The aggressive swarms established their own colonies in already existing colonies kept in the box hives. Details relating to the queen status and the strength of such invaded colonies were collected. Data pertaining to total number of invasions, the dates of final invasion, commencement of drones activity and start of oviposition were gathered. In addition, in situ observations were recorded to document the progress and after effects of intercolony conflict from the time of commencement of aggression until the establishment of a new colony by the aggressive swarm.

The consequence of sequential aggressive swarming was studied in one colony kept in a box hive placed at the nest congregation site. Data on the number of aggressive swarms involved in the sequential attack, respective duration of their attacks, number and duration of invasions and mobbing flight duration of the evicted bees of the invaded colony were collected.

3.3.2. Emergency queen rearing

Emergency queen rearing was studied in four strong, dequeened colonies. Observations pertaining to construction of fake queen cells and appearance and number of emergency queen cells built were taken. The number of gynes emerged from emergency queen cells was also recorded and the percentage of gyne emergence was worked out. The developmental duration of gynes was also found out by working out the time gap between dequeening and emergence of gyne from the emergency queen cell.

3.4. Hiving feral colonies

During the study period 40 feral colonies of stingless bees were transferred from wall cavities into box hives made of either wood or card board. The colonies were captured from the

stone wall cavities of two roofless dilapidated buildings at Bhavanisagar and Maranoor (Plate 2). A short iron rod with a sharp end was used to break the stone wall. The stones were judiciously removed to expose the nest. The various nest components *viz.*, entrance tube, pollen pots, honey pots, brood clusters and resin dumps were totally transferred one after another.

A thorough search was made in different parts of the nest to locate the queen. The site of occurrence of queen inside the nest was also recorded. Later the queen was gently removed along with a piece of brood cluster and transferred into the hive. Resin was smeared both inside and outside the entrance hole of the hive to lure the evicted bees into the hive. The colonies thus captured were shifted to the meliponary after dusk. During some occasions the queen was not located. Appearance of sealed false queen cells either on third or fourth day after hiving was taken as an indication for queenlessness. A strong colony was dequeened to determine the time taken for the origin of such fake queen cells. Similarly in a queenless split the sign of queenlessness was studied. The colony capturing experiences were meticulously recorded for all the hived feral colonies and tips useful for capturing feral colonies from wall cavities were formulated.

3.5. Requeening of orphan colonies

The effectiveness of three different methods of requeening was studied by using either queenless feral colonies or queenless splits specifically made for this purpose. All the orphan colonies which were desperately in need of queen and built many false queen cells were selected for requeening. Queen cells (Plate 3), gynes (Plate 4) and laying queens (Plate 5) were the three queen sources utilized for requeening. The success of requeening was determined by the commencement of provisioning and oviposition process in the dequeened colony.

Queen cells of varying age were directly introduced into five queenless splits of moderate strength. After introduction the colonies were kept at a nest congregation site to ensure

mating. Observations on emergence of gynes from queen cells and commencement of egg laying were taken as indicators to judge the successful acceptance of queen hatched out from queen cells.

Gynes of known and unknown age were directly introduced for requeening six and five queenless splits respectively and kept either at a meliponary or in nest congregation area or in one instance in an isolated place. Gynes needed for this study were collected either from feral colonies or colonies kept in the meliponary or gynes reared from emergency queen cells. The success or failure of gyne introduction was predicted by observing the commencement of oviposition in all the orphan colonies. In addition, the exact time gap between the gyne emergence and commencement of provisioning and oviposition process was found out wherever the gynes of known age were used for introduction.

Requeening of queenless split or orphan colonies was done by introducing a laying queen got from either a weak colony or by dequeening a queen right colony. Two types of queen introduction *viz.*, direct introduction and honey smeared queen introduction were tried to find out any antagonism shown by the workers towards the introduced alien queen. The success of queen introduction was judged by the appearance of new advancing front and commencement of egg laying in the newly built brood cells.

3.6. Foraging behaviour

3.6.1. Pollen foraging

Pollen foraging behavior of field bees of *T. iridipennis* was studied in the aonla (*Emblica officinalis*) germplasm bank maintained at forest nursery at Bhavanisagar. The flowering lasted for 16 days in six varieties from February to March 2008. However, in BSR-1 the flowering continued for one more week. There were two nest aggregations of stingless bees very close to the study site (100 m) consisting of 120 feral colonies. The pollen foraging behaviour was

studied on seven aonla varieties viz., BSR-1, Chakaiya, Kanchan, NA-7, Krishna, Neyveli and Sadivayal maintained in the nursery (Plate 6). Insitu observations pertaining to following parameters were taken up to study the foraging behaviour of *T. iridipennis* on the above aonla varieties.

3.6.1.1. Foraging time

The time of commencement of foraging activity of *T. iridipennis* was recorded from 20.02.2008 to 02.03.2008 on BSR-1 variety and time of cessation was observed on Neyveli variety. BSR-1 and Neyveli varieties were preferred for this study because peak foraging in the fore noon and after noon respectively were found in these varieties. The time of landing of the first bee on the inflorescence was recorded as the time of commencement of foraging. Similarly the time of departure of the last bee observed on the inflorescence was noted as the time of cessation of foraging.

3.6.1.2. Floral handling time

Floral handling time on various aonla varieties was worked out by observing the time spent by a forager with partially loaded corbiculae on a single male flower for pollen collection. Floral handling time of *T. iridipennis* was also worked out on just opened and fully opened flowers by three different kinds of foragers viz., bees with empty corbiculae, bees with half loaded corbiculae and bees with fully loaded corbiculae in BSR-1 variety.

3.6.1.3. Inflorescence handling time

The time spent by a forager on a single inflorescence was found out by using a stop watch on the different aonla varieties.

3.6.1.4. Floral visitation rate

The floral visitation rate on different aonla varieties was computed by counting the number of male flowers visited per minute by a forager.

3.6.1.5. Inflorescence visitation rate

Inflorescence visitation rate on various aonla varieties was calculated by counting the number of inflorescences visited per minute by a forager.

3.6.1.6. Pollen loading time

A forager landing on a flower with empty corbiculae was continuously watched until the corbiculae were totally loaded with pollen. The time gap between first landing and final take off to the nest was found out by using a stop watch to compute the pollen loading time on both just opened flower and fully opened flower. The number of inflorescences visited for fully loading the corbiculae was also counted. BSR-1 variety that was planted very close to the nest congregation site was chosen for this study. Ten such observations were taken.

3.6.1.7. Peak foraging activity

The total numbers of pollen foragers on male flowers were counted at hourly interval from 06.00 a.m. to 06.00 p.m. on three marked inflorescences of similar age in each variety. Foragers with half loaded corbiculae were selected for observation because they alone foraged steadily for some time than the bees with either empty corbiculae or fully loaded corbiculae.

3.6.2. Resin foraging

Resin foraging behaviour of field bees of *T. iridipennis* was studied on three plants viz., mango (*Mangifera indica*), jack (*Artocarpus heterophyllus*) and peepul tree (*Ficus religiosa*). The strategy followed for resin foraging and mean time spent for resin collection during each foraging trip from the above three sources were recorded by making 20 observations at each

source. The resin foragers were paint marked to ascertain if they make repeated visits for resin foraging.

CHAPTER IV

RESULTS

The results obtained during the present investigations are given below.

4.1. Nest architecture

Forty feral colonies were captured from wall cavities of two roofless, dilapidated buildings at Bhavanisagar and Maranoor. Observations taken on the nest architecture during nest removal are presented below.

4.1.1. Nest entrance architecture

A distinct entrance tube of varying length (Table 2) was observed invariably in all the feral colonies hived from wall cavities. On undulating wall surface the length was maximum (9.0 cm). On plain walls the mean length was 1.7 cm. The diameter of the entrance tube was ranging from 0.5 to 1.2 cm (Plate 7).

Number of bees guarding the entrance was directly proportional to the entrance diameter. A maximum of eight guard bees was found in one nest. The number of guard bees was less and the bee traffic was poor in queenless colonies. An internal tunnel was always found in all the nests and the length of it ranged from 5.0 to 14.0 cm (Plate 7).

4.1.2. Distribution of resin dumps

Out of 40 colonies captured resin dumps were found in 35 colonies and they were totally absent in five colonies. Resin dumps were usually found near the internal tunnel (Plate 7) and distributed in more than one place. In 60 per cent of captured colonies the resin dumps were located at two to three sites (Table 3).

4.1.3. Relative distribution of food pots

Out of 40 colonies, in half of the colonies pollen pots and honey pots were separate whereas in nearly one fifth of colonies food pots were adjacent to each other (Plate 8). However, in 27.5 per cent of colonies both the pots were intermixed (Table 4). In a few locations the food pots and brood nest were found in two adjacent cavities linked by a narrow passage way.

4.1.4. Distribution and attachment of brood clusters

Brood nest was totally absent in orphan colonies. In more than half of the colonies the brood clusters were found in two separate groups (Plate 9). Rarely they were also found in three groups. In nearly 50 per cent of the colonies the brood cell clusters were originating from the floor of the cavity (Table 5). In some locations they were also suspended from roof (Plate 10).

4.2. Drone congregations

4.2.1. Nest associated drone congregation

Results pertaining to nest associated drones activity at nest congregation sites are presented in Table 6 and Plate 11. The activity of drones was found from October-2007 to March-2008. Normal swarms led by gynes that occupied the vacant nesting space available in a nest congregation area always attracted drones. In front of such new nesting sites the flight activity of drones was found for a period of three to five days. In one occasion drones were found flying usually in front of the nest of a newly settled swarm for the second time for a period of four days. Drones were also found flying unusually in front of a hive housing a queenright colony. The activity of drones persisted in front of that colony for a period of 19 days even after the removal of queen, two days after the arrival of drones. Similarly a drone swarm was found to exist for four days in front of a hive occupied by an aggressive swarm even after the removal of the gyne a day after its arrival. Drones were observed for eight days in front of a hive once occupied and subsequently deserted by an aggressive swarm.

Observations taken on drone flight behaviour in relation to weather conditions revealed that rains had an adverse influence on flight of drones and caused their disappearance from the congregation area. However, the drones resumed their flight activity after the cessation of rains. Similarly heavy wind also interfered and altered the normal diurnal flight pattern of drones. Drones got scattered and could not fly closer to nest entrance due to strong winds.

The drones were found to stay on twigs or leaf under surface in group during night. Such sleeping clusters remained motionless during night and resumed their activity only after dawn. Few drones were also found to sip nectar from the flowers of *Tecoma stans*, an ornamental shrub. In some of the flying drones their gasters were inflated and inter segmental membranes were visible as white bands.

4.2.2. Non-nest associated drone congregation

Drones were found flying in large groups near the tree trunks of neem and jack found closer to the nest congregation area. In addition, they were also found flying in thousands closer to the base of the bamboo pole projecting out slightly from the roof of an old building housing several feral colonies (Plate 1). The time of commencement of such flights widely varied. The flight activity started between 9.15 to 11.30 a.m. in the forenoon and between 2.30 to 3.00 p.m. in the afternoon. However, the drones stopped flying at the earliest by 5.30 p.m. and at the latest by 6.10 p.m. (Table 7). Such flight activity persisted for one to ten days. Drones were found flying most of the time and landed on the trunks for a shorter time for taking rest. Later they resumed their flight activity.

4.3. Colony reproduction

4.3.1. Swarming

4.3.1.1. Normal swarming

Variations in colony number at nest congregation area

The variations observed in the colony number at the nest congregation area over a period of seven months are given in Table.8. There were 67 colonies to start with, out of which three colonies deserted. Subsequently 34 new colonies arrived out of which only 23 colonies settled in the wall cavities of the old building.

Normal swarming sequence

Swarms were not attracted to trap hives. Both bamboo node hives and well cleaned box hives smeared with resin inside were ineffective in attracting the swarms. Incidentally one swarm settled in one uncleaned empty box hive once used for hiving a stingless bee colony. The hive was left as such in the meliponary and had cerumen pillars, connectives and pollen pots in the advanced stage of decomposition. The swarming sequences and colony establishment were studied in this hive. The various events observed in the process of settling of the swarm are given in the Table 9.

4.3.1.2. Aggressive swarming

Normal aggressive swarming

Observations pertaining to colony founding by aggressive swarms are presented in Table 10. Aggressive swarms were found to attack both populous and weak colonies. Weak colonies were invaded once and aggressors killed all the hive occupants including the queen. A strong colony was invaded four times over a period of six days. Such repeated invasions weakened the colony and resulted in total death of the invaded colony.

The sequence and nature of fighting observed are narrated below. Fierce fighting was observed between the colony occupants and alien invaders. Guard bees actively defended the nest against intruders initially at the nest entrance (Plate 12). They captured the intruders using their mandibles. The fighting pairs fell down on the ground and continued their fight until they were grabbed and carried away by ants. Mandibles were mainly used as fighting tools to mutilate the body, ambulate legs, bite off wings, cause injuries on antennae and lapping tongue and cut off body regions. Sometimes the invaders easily slipped into the hive through the gaps found in between the hive and the lid. Bees fought with each other in pairs to start with inside the hive. Later more number of bees got involved in the fight. As a result a small cluster of fighting bees was formed. All the bees in the cluster died finally by mutually attacking each other. Several hundreds of dead bees and mutilated body parts were found inside the invaded hive. All the intranidal activities including waste disposal and dismantling and removal of empty cocoons were stopped because of fighting among hive bees and intruders (Plate 13). Nest cleaning activities were started only after the nest was brought under the total custody of the aggressive swarm. All the accumulated dead remains of the bees, garbage in the waste dump and dismantled empty cocoons were removed. During the last invasion the gyne was found to lead the aggressive swarm. The bees in the invading swarm brought a large quantity of resin in their legs and they subsequently used it for sealing the gaps.

Arrival of gynes was found to trigger intense hovering of drones in front of the hives occupied by the aggressive swarm on the same day. Such flight activity of drones lasted for five to eight days after the completion of final invasion. The gyne was always found actively moving and often fluttering her wings in the vicinity of internal tunnel. The mated gyne started egg laying within a period of 9 to 13 days (Table 10).

Sequential aggressive swarming

A strange phenomenon of sequential aggressive swarming was observed in a box hive housing a queen right colony, kept at a nest congregation site (Table 11). These aggressions lasted nearly for a month. During this period five aggressive swarms settled one after another in the box hive by killing the previously settled aggressive swarm. The duration of invasion ranged between three to six days. Three to four such invasions occurred in sequence that resulted in total death of the colony. During preliminary invasions the young bees of the invaded hive flew out to escape from the attack of invaders. The frightened bees were found flying in a group far away from the hive in a zig zag fashion. The bees participating in mobbing flight (Plate 14) returned to their hive after about one and half-hours. The final invasion that lasted for nearly three hours resulted in the total dissolution of the invaded colony. The activity of drones was intense and persistent through out the period of ephemeral settling of these aggressive swarms.

4.3.2. Emergency queen rearing

Emergency queen cells were clearly visible in the queen deprived colonies nearly a fortnight after dequeening. Such dequeened colonies built emergency queen cells ranging from six to eleven. These cells were larger than the workers cells and resembled a miniature peanut pod (Plate 15). Initially a prominent constriction was found at one end that later disappeared due to restructuring of the queen cell. The mean developmental period of gynes developing inside the emergency queen cells was ranging from 47 to 51 days and 100 per cent gyne emergence was recorded (Table 12).

Sometimes the bees also built and linked an auxiliary cell to the introduced queen cell (Plate 16). But that auxiliary cell was dismantled subsequently after a day when the colony was given with a small brood cluster having a few larval brood cells. Later the bees constructed and linked an auxiliary cell to one of the larval brood cell (Plate 17).

4.4. Hiving feral colonies

4.4.1. Site of occurrence of queen

The functional queen was mainly located either on or inside the brood nest in 80.0 per cent of the captured colonies (Table 13). The queen was most commonly found on the advancing front. Rarely the queen was also found among young bees or near food pots. Colonies without advancing front usually lacked a functional queen. In such colonies either gynes (Plate 4) or emergency queen cells (Plate 15) were present.

4.4.2. Signs of queenlessness

Initially fake queen cells were built in all queenless colonies (Plate 18). Such fake queen cells construction was observed in captured colonies whenever the queen was lost or killed during the hiving process. Depending on the strength of the colony queen cells construction was observed either on the second or third day. Usually in dequeened colonies and queenless splits queen cell construction was started on the second day. A few sealed fake queen cells were noticed on the third day and several such sealed cells were found on the fourth day which was a sure indication of absence of queen.

4.5. Requeening of orphan colonies

4.5.1. Queen cells

Providing of queen cells to orphan colonies was found to be an effective requeening method in four out of five orphan colonies. The queenless colonies became queen right and the queens started egg laying approximately within a period of nearly two weeks (Table 14).

4.5.2. Gynes

Gyne introduction was also found to be an ideal method of requeening an orphan colony. Five out of six orphan colonies were requeened successfully by introducing gynes of unknown age. However, in one colony mating failure occurred as the colony was kept in an isolated place where drones were not available for copulation. Nest associated drone congregation was noticed only in one out five successfully requeened colonies for a shorter period (4½ h) which lasted only for a day (Table 15).

Age of the gyne at the time of induction markedly influenced the commencement of provisioning and oviposition process. One-day-old gyne took 14 to 15 days to start egg laying whereas two days old gynes took 15 to 19 days (Table 16).

4.5.3. Laying queen

The orphan colonies accepted the alien queens without showing any antagonistic behavior. Both honey smeared queens and queens introduced directly were accepted. The requeened colonies started building advancing front and the introduced queen started egg laying within a period of three to four days (Table 17).

4.6. Foraging behaviour

4.6. 1. Pollen foraging behaviour

4.6.1.1. Foraging time

Stingless bees in general commenced their foraging activity late in the morning (6.50 to 7.55 a.m.) and stopped their foraging trips early in the evening (6.24 to 6.34 p.m.) (Table 18).

4.6.1.2. Handling time

Floral handling time of stingless bees on different aonla varieties varied from 7.4 to 18.2 sec. They took more time to handle the flowers of Krishna and lesser time to handle the flowers in Sadivayal (Fig.1). Foragers with half loaded corbiculae stayed longer on flowers to collect pollen than bees with either empty or fully loaded corbiculae both from just opened and fully opened flowers. They spent more time on just opened flowers than fully opened flowers (Fig.2). Similarly the inflorescence handling time needed for stingless bees showed variations ranging from 12.6 to 31.5 sec. Foragers required more time to handle the inflorescence of BSR-1 and less time to handle the inflorescence of Sadivayal (Fig.3).

4.6.1.3. Visitation rate

In general pollen foragers visited more flowers per minute than inflorescences. Floral visitation rate and inflorescence visitation rate were maximum in Neyveli variety and Sadivayal variety. Pollen foragers collected pollen from 5.2 to 7.2 flowers spread on 3.2 to 4.6 inflorescences per minute (Fig.4 and 5).

4.6.1.4. Pollen loading time

The foraging time required for fully loading the corbiculae (Plate 19) with pollen is presented in (Fig.6). The foragers comparatively required more time to collect and load their corbiculae with pollen from just opened flowers rather than from fully opened flowers. However, the foragers handled less number of inflorescences with just opened flowers and more number of inflorescences with fully opened flowers to fully load their corbiculae.

4.6.1.5. Peak foraging activity

Intense stingless bee activity was noticed on aonla varieties from 8.00 to 9.00 a.m. and 5.00 to 6.00 p.m. There was a lull in the activity of stingless bees from 11.00 a.m. to 3.00 p.m. Of all the varieties, in BSR-1 more bee activity was observed from 8.00 to 9.00 a.m.

During evening hours (5.00 to 6.00 p.m.) the bees were found in abundance in Neyveli variety. In general the bee activity was very low in Sadivayal variety (Fig.7).

4.6.2. Resin foraging behaviour

Foragers actively collected resin oozing out from the cut surfaces of branches and stem of mango trees (Plate 20). Resin foragers came in large number to gather the resin copiously flowing out from the injured bark of peepul tree. In jack the main resin source was the over ripened fruits with cracks and resin flowing out in abundance from stalk after plucking the leaves found on it (Plate 21). Workers in large group collected the resin coming out from the cracks on the fully matured fruit and cut surface of the fruit stalk. Intense resin foraging was observed on fallen ruptured and decomposing fruits. The foraging time required for resin collection is given in Table 19. The foragers spent more time to collect jack resin than from other resin sources. The time needed for resin collection was less in mango.

CHAPTER V

DISCUSSION

The results obtained are discussed below.

5.1. Nest architecture

Stingless bees are cavity-nesting bees like commercially reared honey bees. They especially build their nests in tree cavities and wall cavities. Nest congregation is more common in wall cavities than in tree cavities (Swaminathan, 2000).

The presence of a distinct tubular entrance with a resinous rim and active to and fro bee traffic indicate the existence of a live colony at the nesting site. The entrance tube mainly serves as a passage way allowing the entry and exit of bees. Long entrance tubes play a vital role in preventing the entry of rain water and avoiding both interspecific and intraspecific rivalry. In general the width of the entrance opening is related to the colony strength. In other words nest openings are neither too small to interfere with bee traffic nor so large to make defense impossible (Biesmeiger, 1999). The length and diameter of the entrance tube widely varied (Table 2). Such variations could be attributed to the exact location of the cavity inside the wall. Entrance tubes were usually longer in deep-seated nests built in wall cavities because the bees always extended the entrance tube upto or slightly beyond the wall surface. The nest entrance architecture is a species-specific character. In *Trigona* species a prominent entrance tube is always present and a number of guard bees defend the entrance (Wille and Michener, 1973). The entrance tube is mainly made of cerumen and mud. The bees deposit fresh resin at the outer edge of the tube to ward off ants (Camargo, 1984; Wittmann, 1989). The resinous entrance tube is a recognition mark for the foragers to navigate back into their nest.

The external entrance tube continues inside as an internal passage (Plate 7) leading to the internal parts of the nest. The length of the internal tunnel was determined by the space found in between wall surface and nesting site found inside the wall. The long tunnel mainly helps to defend the colony effectively against intruders. In addition, the sexually matured gyne is often found at the far end of the internal tunnel inside the nest.

Resin dumps were found very near the internal tunnel (Plate 7). The number of resin dumps was varying from one to four (Table 3). Rarely in a few colonies resin dumps were absent. Stingless bees mainly use cerumen, a mixture of resin and wax for nest construction. Guard bees use resin stored closer to internal tunnel as sticky weapons to fight off intruders. Resin bee, *Megachile sp.* that rob resin from stingless bee colonies are attacked by groups of guard bees and the bees inside will daub it with drops of resin until it is entombed. Such an entrapped resin bee was observed in one feral colony at the time of hiving.

Stingless bees do not store their honey and pollen in hexagonal combs as the *Apis* spp do. Instead they construct clusters of oval shaped cerumen pots for food storage. Pollen pots and honey pots were separate in nearly half of the hived colonies (Table 4). The relative distribution of food pots was mainly influenced by the nest volume and number of cavities. If the space availability is more honey pots and pollen pots are either adjacent to each other (Plate 8) or intermixed. Food pots are constructed separately whenever the nesting site consists of small-interlinked cavities. These bees hoard more quantity of pollen than honey.

The brood cells are mostly arranged in horizontal layers forming combs in the genus *Melipona* (Roubik, 2006). However, in *T. iridipennis* brood cells are arranged in loose clusters. Cluster type brood can take advantage of small and irregular cavities. The volume and number of cavities available are the prime factors that mainly determine the number and site of attachment of brood clusters (Table 5). The brood comb consists of advancing front where new brood cells are under construction, dark coloured larval brood with cerumen coat and light coloured pupal

brood without cerumen are attached in chains. The larval brood cells are comparatively heavier than pupal brood cells because they contain larval food and thick resinous coat in addition to larvae.

5.2. Drone congregations

Drones leave their nest two weeks after emergence in *S. postica* and do not return to their nest. Such drones hailing from different nests migrate to form aggregations. These aggregations may be either nest associated or non-nest associated (Engels and Engels, 1984).

Nest associated drone activity is more common and intense especially at nest congregation sites rather than in small meliponaries. Several hundreds of drones hover as a cloud in front of the nest or the site being developed for new nest or box hives having virgin queens. They also produce an audible, dull humming sound while in flight. They usually remain in air for long time without alighting. Sometimes drones also take rest in small groups near the nest (Plate 22) for a brief period and resume their flight activity. They move out of the hovering place whenever an approaching person interrupts their flight activity. Then they return to the same site and resume their flight. Similar type of nest associated drone congregations were also reported in other Trigonine stingless bees viz., *S. posica* (Engles and Englees, 1984). *T. dorsalis* (Roubik, 1990). *Tetragonisca angustula* (Veen van and Sommeijer, 2000) and *T. collina* (drones Cameron *et al.*, 2004). Even drizzling and heavy winds interfere with the flight of drones. However, the drones resume their flight activity after the cessation of drizzling or heavy winds. This findings is in agreement with the finding of earlier workers (Veen van and Sommerijer 2000; Saravanan, 2005).

Drone congregations are formed only during the day time during sunny and warm weather. Drones activity commences only after the dawn and ceases at 6.10 p.m. The dissolution of drones assembly occurs rapidly during the evening. Drones during their out door living

require food for their survival and continuous diurnal flight activity. During the present study drones were found to visit the flowers of *T. stans* for collecting nectar. Sugars present in nectar provide energy to the flying drones. Such type of drone foraging reported in *M. beechii* by Veen *et al.* (1997) and in *M. favosa* by Sommeijer *et al.* (2004) add support to the present finding. They have reported that males regularly depart from the congregation and some drones visit flowers during their departure. Drones of *M. beechii* collect food from flowers for sustaining their life. Hence, it is clear that drones are capable of living independently of their natal nests. During the present study in some of the flying drones the gaster was swollen. The storage of nectar in honey stomach might have caused inflation of gaster and resulted in the exposure of the intersegmental membranes in the gaster region of the drones. A clear drop of sweet nectar came out when the gaster was pressed. This cursory observation strongly confirms nectar foraging by drones.

The activity of drones starts shortly after the arrival of swarm to a new nesting site. Their activity intensified after a short time and continues until the mating of gyne is over. Subsequently the activity declines gradually. The duration of drones activity ranged from 1 to 19 days (Table 6). The duration of drones activity varied with species. In Sumatran stingless bees, *T. laeviceps* the drones activity was noted around the new nest for two days after swarming (Inoue *et al.*, 1984).

Drones are normally attracted to colonies having gynes. Emission of a female sex pheromone from the gyne may be the possible cause for the formation of drone congregations of different durations. The queen pheromone in stingless bee has been identified in *Scaptotrigona postica*. The pheromone is produced in the mandibular glands which contains 2- alcohols and 2- ketones. 2-alcohols attract males from a long distance and 2-ketones trigger copulatory response in males. In addition to female pheromone, some other mechanisms are also involved which attract drone swarms before the nest. Drones may be attracted to even queen right colony, if the

queen of the colony is to be superseded by a gyne. Colonies after gyne removal remained attractive to drones (Table 6) provide support for the above conclusion. Similarly, the ability of an empty hive once occupied and subsequently deserted by a swarm attracting drones for eight days (Table 6) clearly indicated the possible involvement of pheromone residues left by gyne and workers in attracting drones. The activity of drones persists until the gyne mates with a drone. Later there is a gradual decline in the activity of drones that can be mainly attributed to the successful mating of gyne dwelling inside the nest. Such reduced attractiveness of the nest with newly mated queen was reported in *T. angustula* (Veen van and Sommeijer, 2000). Drone congregations probably serve as encounter sites where the males wait for virgin gynes as the mating in stingless bees occurs outside the nest. The gyne enters such a drone aggregation and mates with any one alien drone. Hence, out breeding can be achieved and sibling mating can be avoided by the migration of drones. Drone congregations are always found in proximity to nests in Trigonine stingless bees as they are weak fliers.

Drones of stingless bees also form congregations away from the nest. Such non-nest associated congregations occur on of the base of live tree trunks, bamboo pole (Plate 1) and twigs (Plate 23). Shade availability and proximity to nest congregation area are the major factors that determine the site of congregation. The duration of drone congregation varies from one to ten days (Table 7). The time of commencement and time of cessation of drones activity and flight duration widely varied. The flying drones also take rest at times. Such kind of non-nest associated drone congregation in *T. iridipennis* was also reported by Saravanan (2005) on twigs and dried leaves. Similarly, a large non-nest associated drone congregation in *M. favosa* in the Tobago islands was reported by Sommerijer and Bruijn, (1995). However, they have reported that mating did not occur at these drone congregation areas. Hence, the significance of such non-nest associated drone congregations in not clear.

5.3. Colony reproduction

5.3.1. Swarming

5.3.1.1. Normal swarming

Trap hives kept in nest congregation area for attracting swarms during the present investigations were ineffective. The box hives and bamboo node hives used for trapping were not chosen by the swarms for settling probably because of the following reasons.

- The hives were new and not previously occupied by a colony.
- The box hives were not having bee tight lids.
- Trap hives were not kept in shade.
- Availability of natural nesting sites in the nest congregation area.
- The hives do not mimic the natural nesting sites.

However, in contrast to our observations the trap hives were found to be effective in luring the swarms of *Tetragonisca angustula* (Ratnieks and Hart, 2001). Out of 34 new swarms arrived in the nest congregation area, 23 swarms established successfully (Table 8) which clearly indicated the inclination of swarms to settle in natural nesting sites available in the nest congregation area. Inoue *et al.* (1984) reported that long-term availability of good nesting sites in the nest congregation area often attracted new swarms to such sites. During the present study one swarm successfully settled in a box hive once occupied and deserted by a stingless bee colony containing various nest components *viz.*, entrance tube, internal tunnel, cerumen pillars, connectives and damaged pollen pots (Table 9). Similarly we also observed new swarms to settle in old nesting sites from where feral colonies were removed. Both these observations are in concordance with each other.

Scout bees play a vital role in nest site selection. The odour of the cerumen present in a cavity attracts scout bees. The resinous materials present might mean economy in labour and resources (Inoue *et al.*, 1984). Later the workers take up the site cleaning work. It is followed by the construction of honey pots and pollen pots. The workers transport pollen and honey from mother colony that is a sign for the commencement of swarming process. The swarm led by gyne

arrives after the completion of the above nest site preparatory works. Males hover around the hive entrance after the arrival of swarm. The gyne mates with a drone successfully and starts egg laying.

Colony reproductions in stingless bees mainly occur through swarming. The swarming process in stingless bees is totally different from honey bees. In honey bees the primary swarm is led by old queen and the swarm move far away from the old nest. But in stingless bees the swarms are always led by gynes and establish new daughter nests nearer to the mother nest. Swarm clusters formation does not occur at the time of swarming. Further, the separation of daughter colony from mother colony is gradual. The daughter colony maintains its contact with filial nest for a long time. Hence, swarming occurs slowly in stingless bees that last for several months. Swarms often settle closer to the mother nest because of two reasons. The scout bees always select the nesting sites within their flight range. The sharing of resources *viz.*, building materials and food between mother and daughter nests can easily occur only when the nests are nearer to each other. This kind of swarm settling and their tendency to remain for a long time at location site often give rise to nest congregation.

5.3.1.2. Aggressive swarming

The swarms of *T. iridipennis* also establish new colonies by evicting an already existing natural colony or a colony kept in a meliponiary (Plate 24). Feral colonies are less susceptible to the attacks of aggressive swarms because the colonies are safely located inside the natural cavities. Inadequate availability of nesting site in a locality or a meliponiary is the main cause for the occurrence of such aggressions. Usually the aggressive swarms attack and kill weak colonies. Even strong colonies are killed by sequential invasions. Such attacks also lead to the total stoppage of intranidal activities which results in the non-clearing of waste dump and empty cocoons. An aggressive attack often triggers an escape response in hive bees that fly out of the hive and flew in group. Such mobbing flight lasts for nearly one and half-hours (Table 11).

Similar mobbing flights have been observed in *S. postica* due to the entry of intruders into the hive (Balestieri *et al.*, 2002).

Aggressive swarms also occurred in sequence in a box hive (Table 11). Intense activity of drones was commonly observed in a hive forcibly occupied by an aggressive swarm. The origin and fate of normal and aggressive swarms is given in Fig. 8. Such aggressive swarms always resulted in colony loss. Hence, aggressive swarming must be prevented. The techniques of managing aggressive swarms are presented in Fig. 9.

The present study has clearly documented aggressive swarming for the first time in *T. iridipennis*. Such aggressive swarming behaviour was previously reported in Australian stingless bee, *T. carbonaria* by Heard (1988) where hundreds of workers fought to death on the ground below the hive either as a consequence of robbing or installing one of their reproductive as queen.

5.3.2. Emergency queen rearing

Stingless bees normally produce gynes both under swarming and supersedure impulse in queen right colonies. Gynes are reared in specially built royal cells that are larger and found among worker cells in Trigonine bees. The larvae that consume larger quantity of food develop into queens. In queenless colonies, queens are reared in specially designed emergency queen cells as in honey bees. However, the method of queen cell construction, larval selection and provisioning differ totally in stingless bees. The present study has clearly established the occurrence of emergency queen production in *T. iridipennis* for the first time. Emergency queen rearing occurs in honey bees when a colony becomes suddenly queenless. The fertilized eggs laid in the worker cells serve as a source for queen replacement. The workers rear new queens in a few pea nut shaped emergency cells hanging down from the face of the comb by modifying a few worker cells. The selected young larvae are fed with copious amount of royal jelly and they

develop into queens. Sometimes inappropriate larval selection often leads to the production of inferior queens.

However, the method of emergency queen production is totally different in Trigonine bees. Queenlessness results in strong socio-behavioural disorganization. In a queenless colony the workers build several queen cells continuously, provision them with larval food and close them. Since such cells do not have any eggs, queens will not develop from such fake queen cells (Plate 18). In such a situation emergency queen production is possible only when a worker larva is allowed to consume the larval food from other worker cell after consuming the food provisioned in its own cell (Darchen and Delage, 1970). In the present study the above mechanism has been found to operate in the production of queens under emergency impulse. An emergency queen is produced by additionally nourishing the grown up larva with extra brood food provisioned in an auxiliary cell that is having linkage with the larval cell housing the larva (Plate 17). The bees build 6 to 11 such cells for rising queens (Table 12).

Similar record of emergency queen rearing in *T. varia* reported by Faustino *et al.* (2002) is in agreement with the present findings. They found out that the larva ingested the additional food through the feeding connection and the cell was restructured to accommodate the extra fed larva. However, in the present study such dismantling of auxiliary cells could not be observed. The larval cells together with the auxiliary cells have been remodified into queen cells

5.4. Hiving feral colonies

Special attention was paid to hive populous feral colonies. Such strong colonies showed the following characters.

- Long entrance tube.
- Wider nest entrance to permit simultaneous entry and exit of several foragers.

- More number of guard bees engaged in defending the nest.
- Active bee traffic
- Emission of an audible buzzing sound produced by fanning bees.

Nest removal is comparatively easy from wall cavities. Hence, in the present study feral colonies were captured only from wall cavities found in two dilapidated buildings harbouring several stingless bee colonies (Plate 2). Nest exposure often results in evoking aggressive defense behaviour in stingless bees. The disturbed bees fastly fly out of the nest in group to attack the intruder. They mainly bite the skin of the intruder using their mandibles and also apply resin carried in the mandibles on the skin. They fly into the hairs, ears and eyes and cause nuisance that can be avoided by wearing a bee veil at the time of hiving. Such aggressive attacks are commonly observed while hiving strong colonies and colonies with numerous young flying bees. The disturbed callows fly out their of the nest and started mobbing flight outside the nest (Plate 14) which usually lasts for more than an hour. Since, these bees are not efficient in nest navigation they often drift into neighboring colonies leading to inter colony fights. Through stingless bees lack sting, they adopt multifarious defense strategies to defend their nest. The fire bees, *Oxytrigona* use a caustic secretion containing formic acid. They also emit disagreeable odour from mandibular gland secretions to drive out enemies (Michener, 2000).

The success of hiving feral colonies mainly depends on successfully capturing the queen. Queen mainly dwells in the brood nest. She is often found near the advancing front (Table 13) as her main duty is laying eggs in the newly built and provisioned brood cells found in the advancing front. Queen is the largest bee in the colony and she can be easily recognized by her swollen abdomen with distinct tergites and shorter wings (Plate 5). A colony with advancing front always has a functional queen. The queen can be safely captured along with a small cluster of brood cells by using a small fork

Sometimes the queen cannot be easily located at the time of hiving. However, the absence of queen can be confirmed by the appearance of fake queen cells in queenless colonies. In such colonies queen searching is not needed at the time of hiving. Several fake queen cells are built in contiguous manner which form a district cluster, sometimes these cells are also arranged one above the other (Plate 18). It takes one day for the bees to recognize the absence of queen. The bees make futile efforts to rear a new queen that continues for three months. They start building queen cells over the advancing front on the second day. They complete the construction and seal the cells after provisioning the cells with larval food either on third day or fourth day. Many such sealed queen cells can be seen. Such sealed cells are called fake queen cells as they lack eggs inside. Since the colony is queenless, their oviposition does not occur in the provisioned cells. Such cells are sealed prior to oviposition. Normally queen cells are built during the time of swarming and supersedure. But such cells are few in number and always contain the life stages of queen. These cells are scattered in the brood nest and their resinous coat is removed off to expose the pupal cocoon after the completion of the larval stage. However, such resin removal does not occur in fake queen cells and they remain as such. In course of time the larval food provisioned inside the fake queen cells gets dried up and becomes powdery.

Queenlessness leads to population depletion and finally results in colony loss (Saravanan, 2005). Cell construction and provisioning pattern varies between queen right and queenless colonies. The workers build many queen cells, provision them with larval food and subsequently demolish them after some days in *Plebeia saiqui* (Imperatriz-Fonseca and Oliveira, 1976). Under orphanage the strongly caste interactive nature of the provisioning and oviposition process gets substantially altered and several unusual features appear. Unusual cell construction rhythms, uncommon patterns of cell provisioning and cell sealing are observed in *Geotrigona mombuca* (Lacerda and Zucchi, 1999). The present findings related to the behaviour of queenless colonies is in consonance with the above two findings.

Tips for successful hiving

The tips to be followed for successful capturing of feral colonies at various stages of hiving process from wall cavities are presented below based on the practical experience gained while hiving 40 feral colonies of stingless bees.

General tips

- 1) When colonies are found inside a wall one above the other, hive the top colony first followed by other colonies in a descending fashion.
- 2) Close the nest entrances of other colonies those are in close proximity to the colony to be hived on the previous day night.
- 3) Use a hive of appropriate size for capturing feral colonies. The hive size should be match with the bee population of the feral colony.
- 4) Use halved coconut shells or small mud pots for hiving very small colonies.
- 5) Use only well cleaned hives for transferring feral colonies.
- 6) Use a bee veil while hiving to work calmly without getting annoyed by the disturbed bees.

Nest exposure

- 7) Dislodge the entrance tube easily and safely by making a few pricks at the site of attachment with a pointed needle (Plate 25).
- 8) Tap the stones around the entrance tube one by one with an iron hammer (Plate 26).
- 9) Select the exact stones to be removed for exposing the nest based on the relative exodus of bees while tapping different stones.
- 10) Remove the stones judiciously while exposing the nest to prevent the disorientation of the foragers to the neighboring nests.
- 11) Remove the mortar around the boulders by using a short, thick, sharp ended iron rod.
- 12) Lift and shift the stones carefully to which the nest components are attached.

- 13) Remove and shift the large stones especially found at greater height with great care to avoid any damage to nest components.
- 14) Avoid soiling of nest components while removing the stones by using either a card board sheet or hive lid as a cover.
- 15) Exercise enough care while exposing the nest whenever the back end of the colonies are adjacent to each other to avoid infighting between bees.

Removal of nest components

- 16) Place the removed stones containing nest components on a plastic sheet and then transfer the components to the hive to minimize the chances of losing the queen while hiving the colony.
- 17) Blow air to drive out bees while cutting the connectives and pillars to remove brood clusters.
- 18) Separate the larval and pupal brood clusters suspended from roof before placing them inside the hive to avoid any damage to pupal brood.
- 19) Place the removed brood clusters inside the hive without altering their orientation.
- 20) Do not keep the leaky or damaged honey pots inside the hive.
- 21) Keep sufficient number of pollen pots in the newly hived colony.
- 22) Store excess pollen pots if any in strong colonies for future use.

Queen capturing

- 23) Search for the queen in the brood nest.
- 24) Use a camel hairbrush dipped in honey or a small fork to pick and transfer the queen safely into the hive (Plate 27).
- 25) Use a pen torch to trace out the queen bee hiding in the dark interior parts of the nest.

- 26) Place a portion of the larval brood very near the widened nest entrance to trap the queen which may some times move into the interior parts of the nest cavity while hiving.
- 27) Capture the queen slowly with great care if the queen moves into zone of honey pots.
- 28) Avoid exposure of the captured queen to prevent balling of the queen by alien bees belonging to neighboring colonies.
- 29) Keep the captured queen safely inside a small plastic container along with a few pollen pots or brood cells until the capturing process is completed (Plate 28).
- 30) Release the imprisoned queen after transferring all the nest components into the hive.

Eviction of bees

- 31) Smear resin both on the outer and inner side the hive entrance to lure the disturbed bees into the hive (Plate 29).
- 32) Smear more quantity of resin on the inner side of the hive entrance to induce quick setting of bees in the hive.
- 33) Smear minimum quantity of resin out side the hive entrance to avoid resin robbing by bees from neighboring colonies.
- 34) Refix the removed entrance tube to the hive entrance to ensure quick settling of bees (Plate 30).
- 35) Close the exposed feral nest with soil to prevent the bees from reentering into their feral nest.
- 36) Place the hive at the same height so that all the incoming foragers of the feral colony enter into the hive.
- 37) Use a bird feather to remove safely the flightless young bees found crawling on the stones into the hive (Plate 31).
- 38) Avoid hiving colonies during windy days which will greatly hinder the settling of bees in the hive.

Care of colonies after capturing

- 39) Seal the glass lid of the hive and make it air tight by smearing resinous material or an adhesive all around the glass plate.
- 40) Shift the colony to a new place a few days after hiving.
- 41) Shift the hives carefully without much jerks and shakes, if the captured colony has to be taken to a new location on the same day.
- 42) Keep the newly captured colonies under shade.
- 43) Provide dummy division board to small colonies based on need (Plate 32).
- 44) Offer pollen, honey, resin and pupal brood for small colonies to ensure faster growth.

5.5. Requeening of orphan colonies

Queen loss may occur sometimes while hiving feral colonies. Such hived queenless colonies also need queens for their survival. Requeening is an important operation practiced both in meliponiculture and apiculture to save queenless colonies. Orphan colonies of stingless bees that are badly in need of queen can be requeened safely by introducing either ripe queen cells or gynes or laying queens. The queen cells introduced as such are totally accepted (Table 14). The bees do not damage the introduced queen cells. Intact ripe queen cells containing mature queen pupae can be safely removed from the brood nest. Such cells do not have cerumen coverings. Instead they have protective silken coverings. They are best suited for requeening. Workers remove the resin coat from the queen brood cells after larval stage is completed. The larva that is ready for pupation constructs a silken cocoon around itself and becomes pupa. A few empty cocoons of workers that are yet to be dismantled and disposed off usually surround silken cocoons of the queens. The gyne bites one or two small holes on the cocoon a day prior to emergence. The hatched out gyne starts egg laying after successful mating and thus the requeening process is completed.

Requeening can be done successfully by introducing gynes (Table 15 and 16). Orphan colonies requeened with gynes do not show any hostility towards the introduced gynes. The gynes after introduction move fast inside the nest components and disappear. Sexually matured gynes are often found near the internal tunnel. They may wait at this site for performing nuptial flight. In general requeened colonies are not very much effective in attracting drone swarm as observed in this study except in one colony (Table 15). The exact reasons for this phenomenon are yet to be explored in detail. However, out of 11 requeened orphan colonies only in one colony drones activity was found for a brief period (Table 15). Drones activity is vital for ensuring successful mating. A few gynes are produced and tolerated in *T. iridipennis* colony either for replacing the old queen or for heading a new swarm in a queen right colony. Other gynes are starved, killed and evicted out after their ephemeral life (Saravanan, 2005). However, in queenless colonies the bees do not exhibit balling or any other antagonistic behaviour towards the introduced gynes.

Laying queens are readily accepted by queenless colonies (Table 17). Direct introduction of alien queens do not induce any non-acceptance behaviour in hive bees. The orphan colonies even readily accepted queen not smeared with honey. Hence, masking the queen smell is not needed for achieving successful queen introduction in stingless bees. However, the introduction of a new queen in a queenless colony triggered some behavioural changes. The bees became excited. They moved closer to the introduced queen for touching her body with their antennae and departed later. Queen introduction is comparatively easier in orphan stingless bee colonies than in honey bee colonies. The direct introduction of queen often results in balling and death of queen in honey bee colonies. Hence, queen cages and special introduction methods are essential in *A. mellifera* (Mc Cutcheon, 2001).

Keeping requeened colonies in isolated places often resulted in mating failure for want of drones in such sites (Table 15). Hence, it is highly essential to keep the requeened colonies in a

meliponary or at nest congregation sites which will pave way for mating and lead to successful requeening. The methods of saving orphan colonies are given in Fig. 10.

5.6. Foraging behaviour

5.6.1. Pollen foraging behaviour

Stingless bees were found to be the important floral visitors in all the seven aonla varieties as there was a nest congregation near the aonla germ plasm bank having nearly hundred feral colonies. They were also equally attracted to *Simaruba glauca* planted adjacent to the nest congregation area. Honey bees were found on aonla only occasionally during this study. Bajpai (1968) reported that *Apis spp.* were found to visit aonla flowers late in the evening after 6.00 p.m. and early in the morning from 5.00 to 6.00 a.m. The reason for the poor activity of Indian bees can be attributed to lack of feral colonies in the study site and availability of other attractive bee forage like *Sapindus emarginates* adjacent to aonla trees. Anthesis took place from 5.00 to 9.00 p.m. in aonla cultivars (Venkatesan, 2006). This may be the possible reason for the occurrence of two foraging peaks viz., evening peak (5.00 to 6.00 p.m.) and morning peak (8.00 to 9.00 a.m.). The stingless bees collected only pollen grains released from male flowers during late evening hours. However, they collected the pollen grains released after the cessation of their foraging activity during the next day morning. A lull in stingless bee activity was found from 11.00 a.m. to 3.00 p.m. This period is ideally suited for taking up any chemical plant protection measures against insect pests infesting inflorescences. The enticement of bees on aonla varieties varied. Production of more number of dense inflorescences and profuse flowering in BSR-1 and Neyveli varieties made them more attractive to stingless bees (Plate 33). Sadvayal variety was less attractive because of the production of few inflorescences with less number of male flowers.

Rains negatively influenced the foraging activity of stingless bees both directly and indirectly. Unusual rains received during January resulted in delayed flowering of aonla. Further, heavy rains washed away the pollen grains and drastically reduced the pollen availability for

bees. Availability of large number of dense inflorescences caused reduction in floral and inflorescence handling time and increased the floral and inflorescence visitation rates. Similarly bees collected pollen grains easily and quickly from fully opened flowers rather than from just opened flowers (Fig.2.) Number of male flowers, extent of flower opening, weather conditions, existence of other competing species of floral visitors and floral sources influenced the pollen availability to stingless bees. The stingless bees also collected honeydew excreted by aphids infesting and colonizing aonla flowers and shoots. A similar mutualistic relationship between plant hoppers and *Trigona sp.* reported earlier confirms the present finding (Carvalho, 2004). The adults of plant hoppers provided greater amount of honeydew to stingless bees than nymphs.

5.6.2. Resin foraging

Unlike honey bees stingless bees, mix resin with wax and use this combination product viz., cerumen for constructing the nest components. Hence, adequate resin availability in a locality is highly essential for colony establishment. Resin is an important component required for designing the external and internal entrances, colony defense and nest orientation. Both pollen and honey stored in resinous pots do not get spoilt because of the antimicrobial properties shown by the resin. Resin foraging is a tough task and even lethal sometimes as some of the resin foragers are trapped in resin while gathering resin (Plate 34). The time needed for gathering resin depends on the consistency, viscosity and gummy nature of the resin. Stingless bees are very efficient in detecting the resin sources and eagerly collect resin from cut surface of branches, barks and cracks found on ripened fruits in groups. Group foraging strategy followed while resin collection is the primary cause for resin robbing. Foragers rob resin from the corbiculae of other foragers (Plate 35) since this kind of stealing is much easier and less time consuming rather than collecting resin oozing out from plant surface. Intra specific resin robbing reported by Saravanan *et al.*, 2004 lend support to present findings.

Future thrust

The present study has brought out some of the hitherto unknown aspects of social biology and colony reproduction in stingless bees. The hiving techniques standardized through this investigation will be very much helpful to ensure colony availability that is a basic requisite needed for popularising meliponiculture. However, the techniques for transferring feral colonies from wall cavities without breaking the wall are to be refined. Aonla has been found to be a potential pollen source for stingless bees. The role of stingless bees in enhancing aonla productivity requires further experimentation. Through this research study has brought out the extranidal behaviour of drones their developmental biology is yet to be found out. Similarly time needed for queen development is yet to be worked out. Intensifying research efforts to fill these research gaps in the years to come will be very much helpful to advance the art and science of stingless bee keeping in India.

CHAPTER VI

SUMMARY

The salient research findings are summarized below.

- ❖ Nest entrance architecture of feral stingless bee colonies varied according to the wall surface and mode of arrangement of stones. The number of bees guarding the entrance also varied depending on the colony strength.
- ❖ Resin dumps in natural colonies were found usually next to internal tunnel. In general pollen pots and honey pots were distributed separately. In some colonies the food pots were either intermixed or adjacent to each other.
- ❖ Space available inside the wall cavity mainly determined the arrangement and distribution of the brood nest. The various brood nest components *viz.*, pupal brood and larval brood with advancing front were found one above the other or in two separate clusters either adjacent or far apart. The brood cell clusters were attached either to the floor or roof or side wall of the cavity.
- ❖ Nest associated and non-nest associated drone congregations were found during day time which lasted for 1 to 19 days and 1 to 10 day respectively. Both rains and winds interrupted the flight activity of drones.
- ❖ Newly settled normal swarms and aggressive swarms led by gynes were more effective in attracting drones.
- ❖ Drones activity was unusually observed in front of a queen right colony, a box hive where an aggressive swarm stayed ephemerally and a newly settled swarm even after the removal of gyne.
- ❖ Drones formed sleeping or resting clusters on the twigs and leaves of jack tree found closer to the nest congregation area.

- ❖ Non-nest associated drones activity was also observed in front of jack and neem tree trunks and a bamboo pole found very near the nest congregation area. The time of commencement and time of cessation of drones activity were erratic.
- ❖ Foraging ability of drones was confirmed by their floral visitation and swollen gaster which was the secret of their survival even after quitting their mother nest.
- ❖ The number of colonies in a nest congregation area over a period was found to be dynamic. The availability of nesting space mainly influenced the settling of new swarms and desertion of settled swarms in the nest congregation site.
- ❖ Empty box hive once housing a stingless bee colony was found to be more effective in attracting a natural swarm. Swarm occupancy occurred after the completion of preparatory cleaning work and construction of food pots.
- ❖ The root cause for aggressive swarming was found to be the lack of nesting space in and around the nest congregation site. A single invasion was enough for an aggressive swarm to kill a weak colony. However, two to three preliminary invasions followed by a final invasion were needed for weakening and killing a strong colony.
- ❖ Repeated aggressive invasions induced mobbing flight behavior in hive bees which lasted nearly for one and half hour for saving their life from such aggressions.
- ❖ An aggressive swarm failed to establish a new colony when it was invaded by another stronger aggressive swarm.
- ❖ Occurrence of such sequential aggressive swarming greatly hindered the colony reproduction.
- ❖ The existence of a queen rearing mechanism under emergency impulse was found out and reported for the first time in *T. iridipennis*. Such emergency queen cells became visible two weeks after dequeening.

- ❖ The worker larva destined to become a queen was additionally nourished with extra quantity of brood food kept inside an auxiliary cell which was linked to the larval cell by a feeding connection.
- ❖ Simple techniques were developed for safe removal of various nest components, young bees and queen while capturing feral colonies.
- ❖ Fixing the entrance tube and or smearing resin around the hive entrance both internally and externally resulted in quick settling of displaced bees while hiving feral colonies.
- ❖ A laying queen was commonly found roaming in the region of advancing front inside the nest.
- ❖ Queenless natural colonies, dequeened colonies and queenless splits built several fake queen cells for rearing queens in vain.
- ❖ Orphan colonies were requeened successfully by the introduction of either ripe queen cells or newly hatched gynes or laying queens. A gyne introduced into a queenless colony became a functional queen two weeks after emergence.
- ❖ Stingless bees were found to be the dominant flower visitors in aonla which mainly offered pollen to these bees as a floral reward.
- ❖ Bimodal foraging activity of stingless bees was found once during morning (8.00 - 9.00 a.m.) and another time during evening (5.00 to 6.00 p.m.).
- ❖ Stingless bees required more time to collect pollen from just opened flowers rather than from fully opened flowers.
- ❖ Stingless bees actively collected resin from damaged fruit stalk, cracks found on over ripened fruit and fallen rotten fruit of jack, pruned branches and damaged bark of mango and damaged trunk of peepul tree.

- ❖ Resin foraging was done usually in group and resin robbing was an unusual strategy of resin collection practiced by few resin foragers.

Fig. 1. Floral handling time of *T. iridipennis* on different aonla varieties.

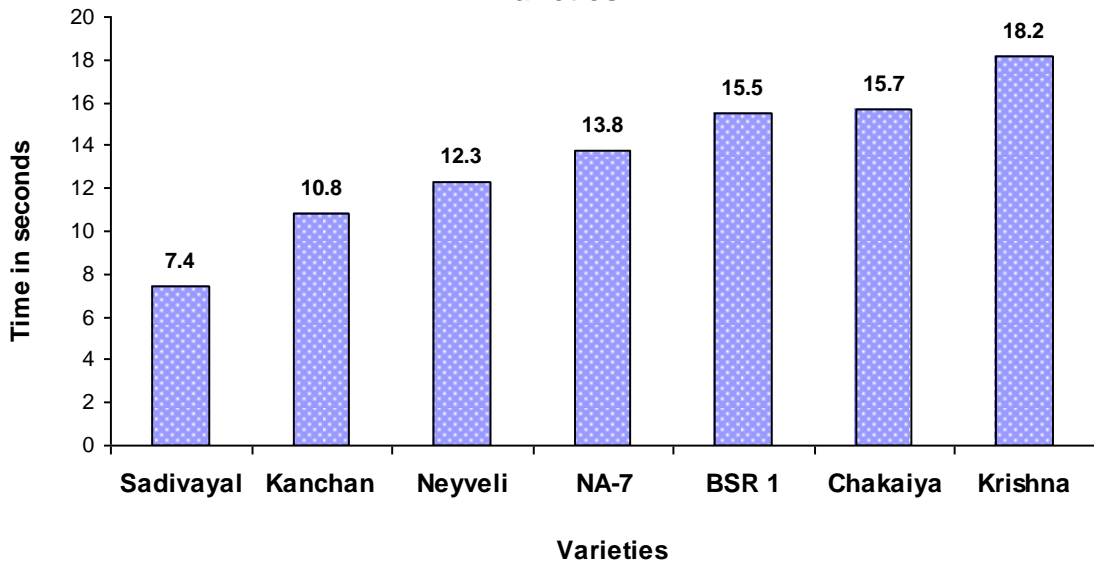


Fig. 2. Effect of extent of flower opening on floral handling time of *T. iridipennis* in aonla variety BSR-1

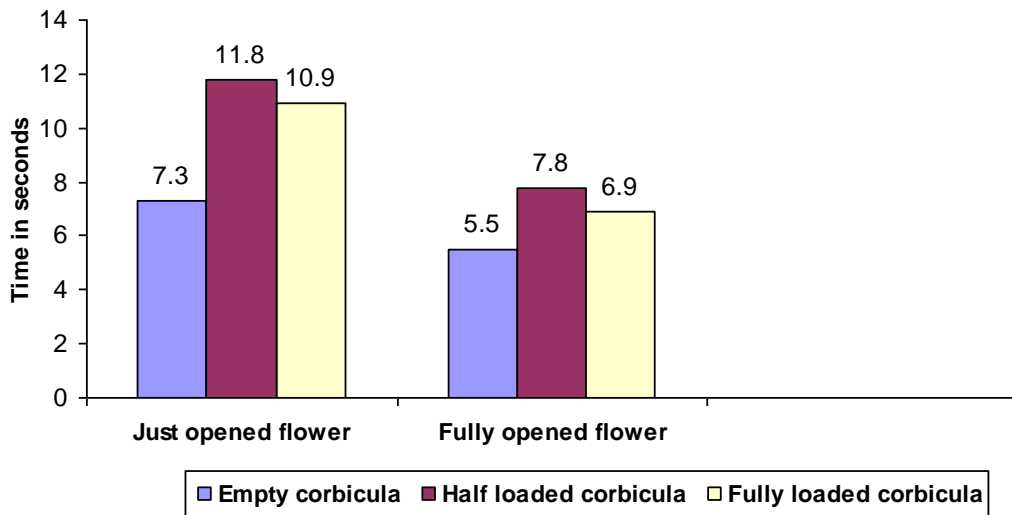
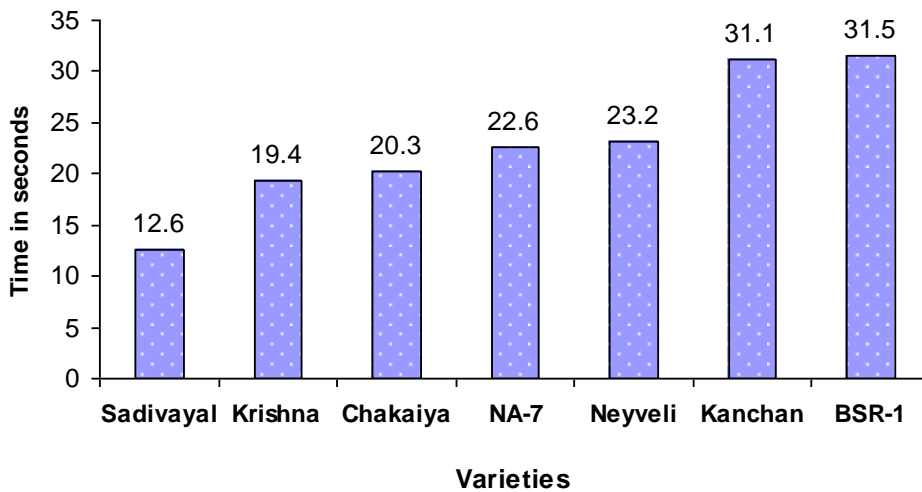


Fig. 3. Inflorescence handling time of *T. iridipennis* on different aonla varieties.



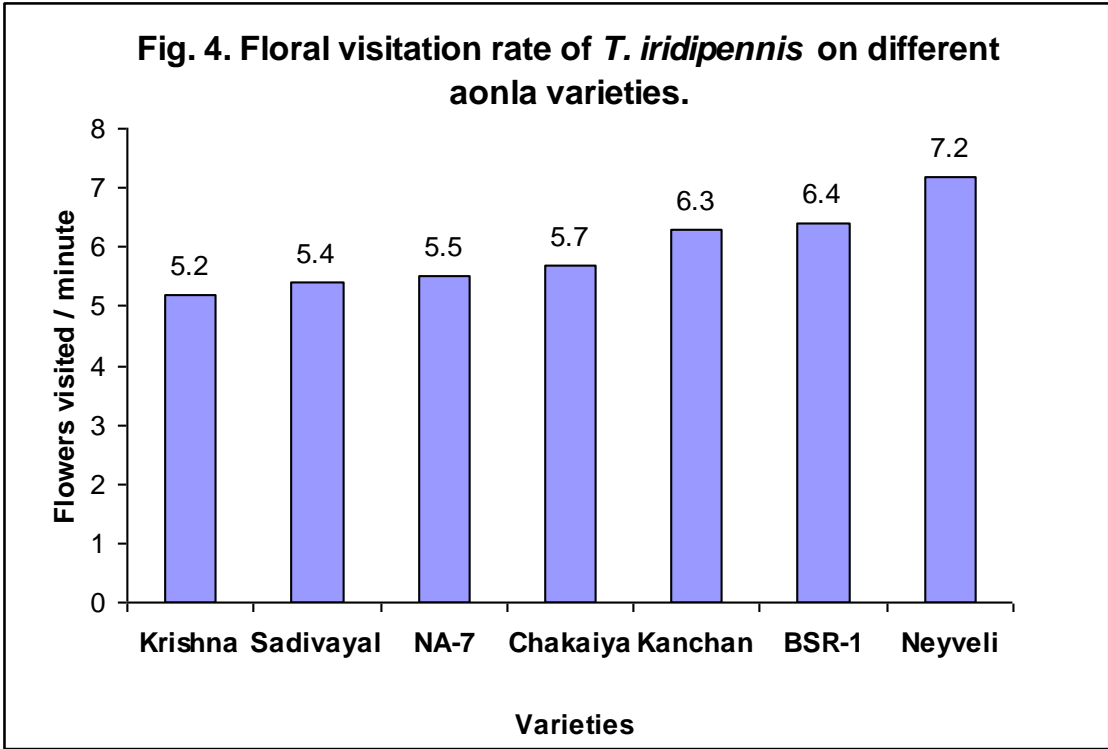


Fig. 5. Inflorescence visitation rate of *T. iridipennis* on different aonla varieties.

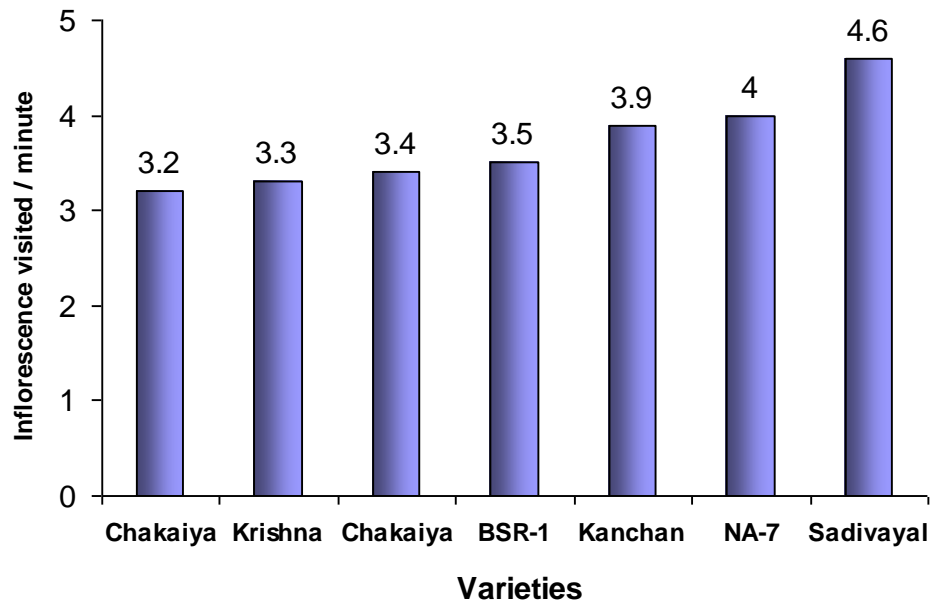


Fig. 6. Pollen foraging time and inflorescence visitation rate for fully loading corbiculae

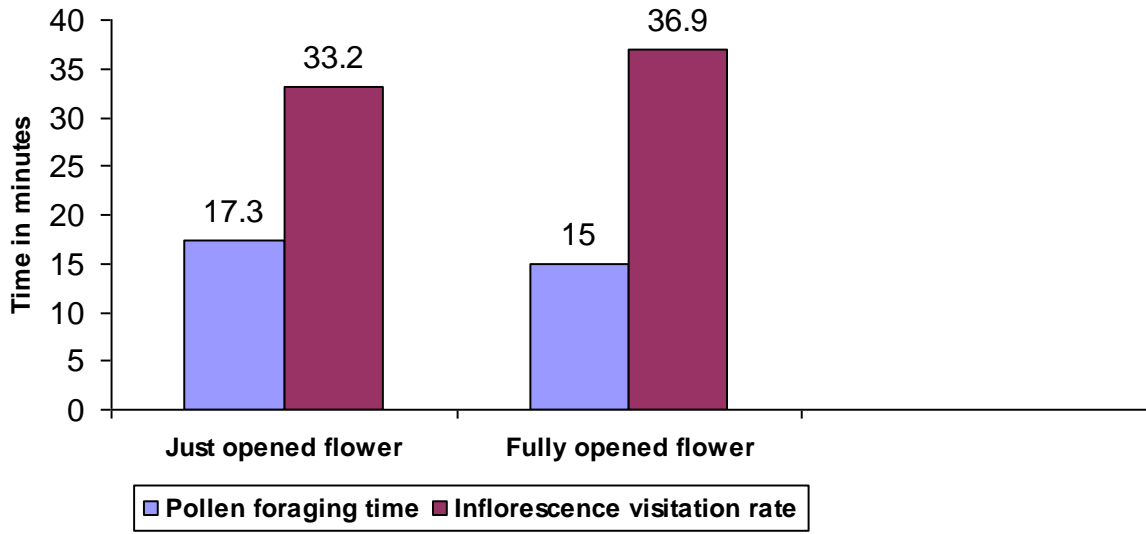
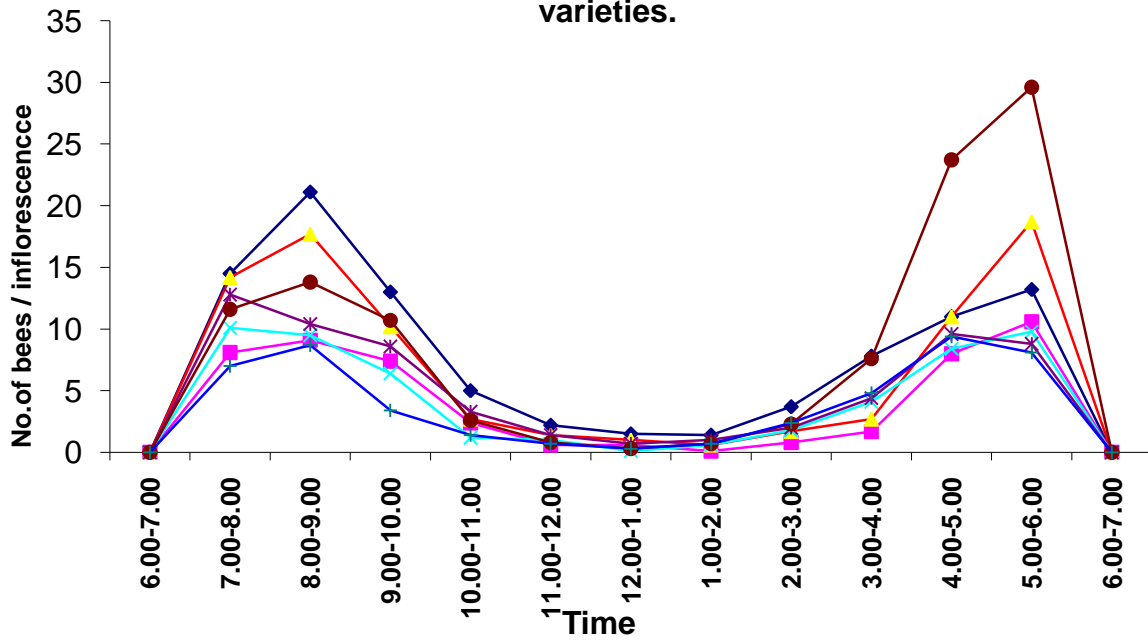


Fig. 7. Peak foraging activity of *T. iridipennis* on different aonla varieties.



◆ BSR-1 ■ Kanchan ▲ NA-7 ✕ Krishna * Chakaiya ● Neyveli + Sadivayal

Table 15. Requeening of orphan colonies with gynes of unknown age

Sl. No.	History of the split colony	Date of introduction of gyne	Date of start of drones activity	Date of commencement of POP	Duration between gyne introduction and POP (Days)
1.	Splitting done during hiving	24.09'07*	Could not be seen	10.10.'07	16
2.	Gyne led colony	26.09.'07*	Could not be seen	-	-
3.	Queenless residual colony	28.11.'07	14.12.07**	18.12.'07	21
4.	Split made of stranded foragers, with pupal brood and food pots	31.01.'08	Could not be seen	11.02.08	12
5.	Split colony with native gyne	12.03.'08	Could not be seen	24.03.08	13
6.	Split colony with introduced gyne	12.03.'08	Could not be seen	18.03.08	7

* Date of capturing of feral colony

** 11.00 a.m. to 3.30 p.m.

NCS: Nest Congregation Site
Oviposition Process

POP: Provisioning and

Table 16. Requeening of orphan colonies with gynes of known age

Sl.No.	Date of emergence of gyne	Date of introduction of gyne	Age of gyne at the time of introduction (Days)	Date of commencement POP
1.	15.03.'08	17.03.'08	2	31.03.'08
2.	15.03.'08	17.03.'08	2	02.04.'08
3.	15.03.'08	17.03.'08	2	29.03.'08
4.	17.03.'08	18.03.'08	1	30.03.'08
5.	17.03.'08	18.03.'08	1	31.03.'08
				Mean

POP: Provisioning and Oviposition Process

Table 17. Requeening of orphan colonies with laying queens

Sl.No	Date of introduction of queen	Mode of introduction	Acceptance of the introduced queen	Date of construction of brood cells	Date of commencement of POP	Time gap between queen introduction and egg laying (Days)
1.	28.10.'07	Honey Smeared	Accepted	30.10.'07	31.10.07	4
2.	05.11.'07	Honey Smeared	Accepted	07.11.'07	08.11.07	4
3.	14.01.'08	Direct	Accepted	16.01.'08	17.01.08	4
4.	24.01.'08	Direct	Accepted	25.01.'08	26.01.08	3
5.	09.04.'08	Direct	Accepted	10.04.'08	11.04.08	3
					Mean	3.6

POP: Provisioning and Oviposition Process

Table 6. Nest associated drones activity in nest congregation sites

Congregation Site I: Feral colonies						
Sl .No.	Date of start	Date of cessation	Duration (Days)	Time of start range (a.m.)	Time of cessation range (p.m.)	Nature of the colony attracting drones
1.	13.10.'07	16.10.'07	4	9.15-10.20	5.25-5.45	Feral colony-Could not be assessed
2.	12.02.'08	15.02.'08	3	9.30-11.00	5.45-5.55	Settling of a new swarm
3.	03.03.'08	05.03.'08	3	9.20-10.30	5.45-6.10	Settling of a new swarm
4.	13.03.'08	16.03.'08	4	8.10-9.15	5.55-6.10	Settled swarm
Congregation Site II: Meliponary						
1.	11.10.07	16.10.07	6	9.15-9.30	5.35-5.45	Settled aggressive swarm
2.	14.12.08	14.12.07	1	11.00	3.15	Gyne introduced colony
3.	31.12.08	04.01.08	5	9.30-11.15	5.35-5.55	Settled aggressive swarm
4.	22.01.08	9.02.08	19	9.15-11.15	3.30-6.05	Queen right colony-Dequeued subsequently
5.	24.01.08	31.01.08	8	9.30-10.15	5.30-5.55	Occupied and deserted by an aggressive swarm
6	28.01.08	04.02.08	8	9.20-9.45	5.35-6.05	Settled aggressive swarm
7	15.02.08	18.02.08	4	8.30-8.45	5.45-6.05	Aggressive swarm made gyneless one day after arrival

Table 11. Establishment and loss of colonies due to sequential aggressive swarming

Sl. No	Dates of invasions	Duration of invasions (Days)	Queen status in invaded colony	No. of invasions		Mean duration of mobbing flight of frightened hive bees (hr.-min.)
				Preliminary	Final	
1.	11.03.'08 to 14.03.'08	4	Queen right	3	1	1.28
2.	15.03.'08 to 18.03.'08	4	Alien gyne	3	1	1.15
3.	20.03.'08 to 22.03.'08	3	Alien gyne	2	1	1.35
4.	26.03.'08 to 31.03.'08	6	Alien gyne	3	1	1.20
5.	01.04.'08 to 03.04.'08	3	Alien gyne	2	1	1.18
	Mean	4.0		2.6	1	1.23

Table 10. Colony founding by normal aggressive swarming

Sl. No.	Invaded colony		No. of invasions	Date of invasions	Drones activity			Date of commencement of POP	Time required for gyne - queen transformation(Days)
	Status of queen	Bee population			Date of start	Date of cessation	Duration (Days)		
1.	Queen right	150	1	31.12.'07	31.12.'07	04.01.'08	5	09.01.08	10
2.	Miniature queen	100	1*	24.01.'08	24.01.'08	31.01.'08	8	-	-
3.	Queen right	750	1	28.01.'08	28.01.'08	04.02.'08	8	09.02.'08	13
4.	Queen right	1100	4**	6.10.'07 to 11.10.'07	11.10'07	16.10.'08	6	20.10.'07	10
5	Queen right	500	1	16.04.08	16.04.08	18.04.08	3	24.04.08	9

* Aggressive Swarm deserted the occupied hive after a brief stay of 6 h

**Preliminary invasions (3) + Final invasion (1)

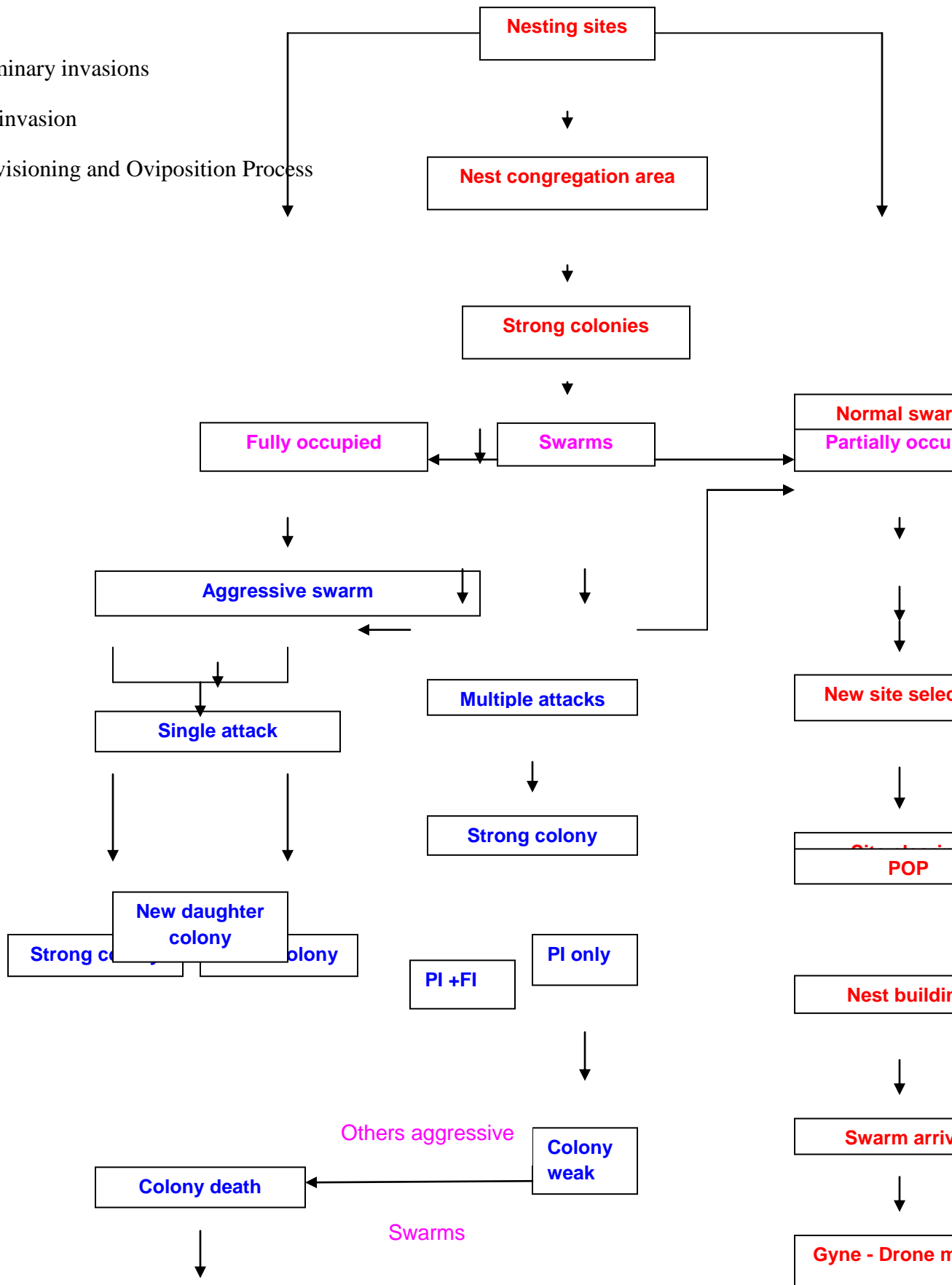
POP: Provisioning and Oviposition Process

Fig: 8 Origin and fate of normal and aggressive swarms in stingless bee

PI: Preliminary invasions

FI: Final invasion

POP: Provisioning and Oviposition Process



Colony replacement



Gyne - Drone mating POP



**Retarded
development**



New daughter c

Table 2. Nest entrance architecture of colonies captured from wall cavities

Sl.No.	Parameter	Maximum	Minimum	Mean*± SE
1.	Length of entrance tube	9.0 cm	0.3 cm	1.7±0.96 cm
2.	Diameter of entrance tube	1.2 cm	0.5 cm	0.8± 0.06cm
3.	Length of internal tunnel	14.0 cm	5.0 cm	8.0±1.00 cm
4.	Number of bees guarding the entrance	8.0	3.0	3.5±0.55

*Mean of 40 observations.

Table 3. Relative distribution of resin dumps

Sl.No.	Parameter	Number / colony	Per cent
1.	Single resin dump	5	12.5
2.	Few dumps (2-3)	24	60.0
3.	Many dumps (>4)	6	15.0
4.	Colonies without resin dumps	5	12.5
	Total	40	100

Table 4. Relative distribution of food pots

Sl.No.	Parameter	Number of colonies	Per cent
1.	Pollen pots and honey pots -Separate	22	55.0
2.	Pollen and honey pots -Adjacent	7	17.5
3.	Pollen pots and honey pots -Intermixed	11	27.5
	Total	40	100

Table 5. Distribution and attachment of brood clusters

Sl.No.	Parameter	Number of colonies	Per cent
A. Distribution of brood clusters			
1.	Single cluster	8	20.0
2.	Double clusters- Adjacent	7	17.5
3.	Double clusters -Separate	23	57.5
4.	Triple clusters	2	5.0
	Total	40	100.0
B. Attachment of brood clusters			
1.	Roof	15	37.5
2.	Floor	18	45.0
3.	Side wall	7	17.5
	Total	40	100.0

Table 13. Site of occurrence of queen inside the nest

Sl.No.	Site of occurrence	Number of colonies	Per cent
A. Brood nest			
1.	On advancing front	16	40.0
2.	On larval brood	6	15.0
3.	Near pupal brood	4	10.0
4.	Inside brood cluster	6	15.0
B. Other nest components			
1	Pollen pots	1	2.5
2.	Honey pots	3	7.5
3.	Among young bees	4	10.0
	Total	40	100.0

Table 14. Requeening of orphan colonies with queen cells

Sl. No.	Date of queen cell introduction	Date of emergence of gyne	Date of start of POP	Time gap between gyne emergence and start of POP (Days)
1.	26.01.'08	28.01.'08	10.02.'08	14
2.	07.02.'08	15.02.'08	29.02.'08	15
3.	13.02'08	06.03.'08	21.03.'08	16
4.	13.02'08	07.03.'08	22.03.'08	16
5.	13.02'08	07.03.'08	*	-
			Mean**	15.25

* Queen loss occurred

** Mean of four values

POP: Provisioning and Oviposition Process

Table 7. Activity of drones at non-nest associated drone congregation sites

Congregation site I: Base of the trunk of a neem tree					
Sl.No.	Date of start	Date of cessation	Duration (Days)	Time of start (a.m.)	Time of cessation (p.m.)
1.	20.10.'07	22.10.'07	3	9-15-9.25	5.30-5.50
2.	14.02.'08	19.02.'08	6	9.15-10.05	5.25-5.45
3.	03.03.'08	03.03.'08	1	11.15	5.50
4.	10.03.'08	10.03.'08	1	11.30	5.30
5.	13.03.'08	13.03.'08	1	2.30	5.45
Congregation Site II: Bamboo pole projecting from a building roof					
1.	14.02.08	23.02.08	10	9.20-10.15	5.35-6.10
2.	26.02.08	28.02.08	3	9.30-10.40	5.55-6.10
3.	03.03.08	04.03.08	2	10.15-11.15	5.45-5.55
4.	12.03.08	12.03.08	1	3.00	5.30
Congregation Site III: Trunk of jack tree					
1.	13.03.08	13.03.08	1	2.30	5.45

Table 8. Variations in colony number at nest congregation area

Sl.No.	Month and year	No. of new arrivals	No. of colonies newly established	No. of new colonies deserted	No. of old colonies deserted	Total no. of colonies
1.	August-'07	-	-	-	-	67
2.	September-'07	4	3	1	-	70
3.	October-'07	5	4	1	1	73
4.	November-'07	4	3	1	-	76
5.	December-'07	5	4	1	-	80
6.	January-'08	4	2	2	-	82
7.	Febraury-'08	5	3	2	1	84
8.	March-'08	7	4	3	1	87
	Total	34	23	11	3	87

Table 1. Basic data on feral colonies found in a nest congregation site

A) Metric data on entrance tube

S. No	Parameter	Maximum	Minimum	Mean*
1.	Length of entrance tube	4.2 cm	0.1 cm	1.3cm
2	Diameter of entrance tube	1.5 cm	0.4 cm	0.7 cm
3.	Height of nest entrance from ground level	3.0 m	0.16 m	1.14 m
4.	Number of bees guarding the entrance	8.0	2.0	3.6

* Mean of 67 observations

B) Distribution of guard bees at the nest entrance

S. No	Range	Number of colonies
1.	2-3	33
2.	4-6	31
3.	7-8	3
	Total	67

C) Variations in the length of entrance tube

S. No	Range	Number of colonies
1.	< 1cm	29
2.	1-2	17
3.	2-3	16
4.	3-4	4
5.	> 4cm	1
	Total	67

Table 9. Sequence of normal swarming

Days after swarm arrival	Date of observation	Events
1	25.03.'07	<ul style="list-style-type: none">• Arrival of scout bees and some workers• Cleaning of interior parts of the hive• Removal of decomposed and powdered pollen• Hovering of drones near the entrance
2	26.03.'07	<ul style="list-style-type: none">• Construction of one honey pot.• Construction of two pollen pots• Inflow of resin into the hive• Continuation of waste removal
6	30.03.'07	<ul style="list-style-type: none">• Progressive construction of pollen pots and honey pots
7	31.03.'07	<ul style="list-style-type: none">• Arrival of swarm
8	01.04.'07	<ul style="list-style-type: none">• Drones flying activity before hive entrance
10	03.04.'07	<ul style="list-style-type: none">• Construction of advancing front
12	05.04.'07	<ul style="list-style-type: none">• Completion of provisioning and oviposition process

Table 12. Emergency queen rearing

Sl.No.	Parameter observed	Colony I	Colony II	Colony III	Colony IV	Mean
1	Date of dequeening	24.01.'08	13.03.'08	8.04.'08	8.04.'08	-
2	Date of commencement of construction of fake queen cells	27.01.08	16.03.08	11.04.'08	11.04.'08	-
3	Date of visibility of emergency queen cells	6.02.08	26.03.08	22.04.08	22.04.08	-
4	No. of emergency queen cells constructed	10	7	11	6	8.5
5	No. of gynes emerged	10	7	11	6	-
6	Gyne emergence (%)	100	100	100	100	-
7	Gyne emergence period	15.03.2008 to 22.03.2008	30.04.08 to 03.05.08	20.05.08 to 23.05.08	21.05.08 to 24.05.08	-
8	Time gap between queen removal and appearance of prepupal stage (Days)	14	14	15	15	14.5
9	Total developmental period of gyne (Days)	52-59	49-52	43-46	44-47	47-51

Table 18. Time of commencement and time of cessation of bee visitation in aonla during February – March 2008

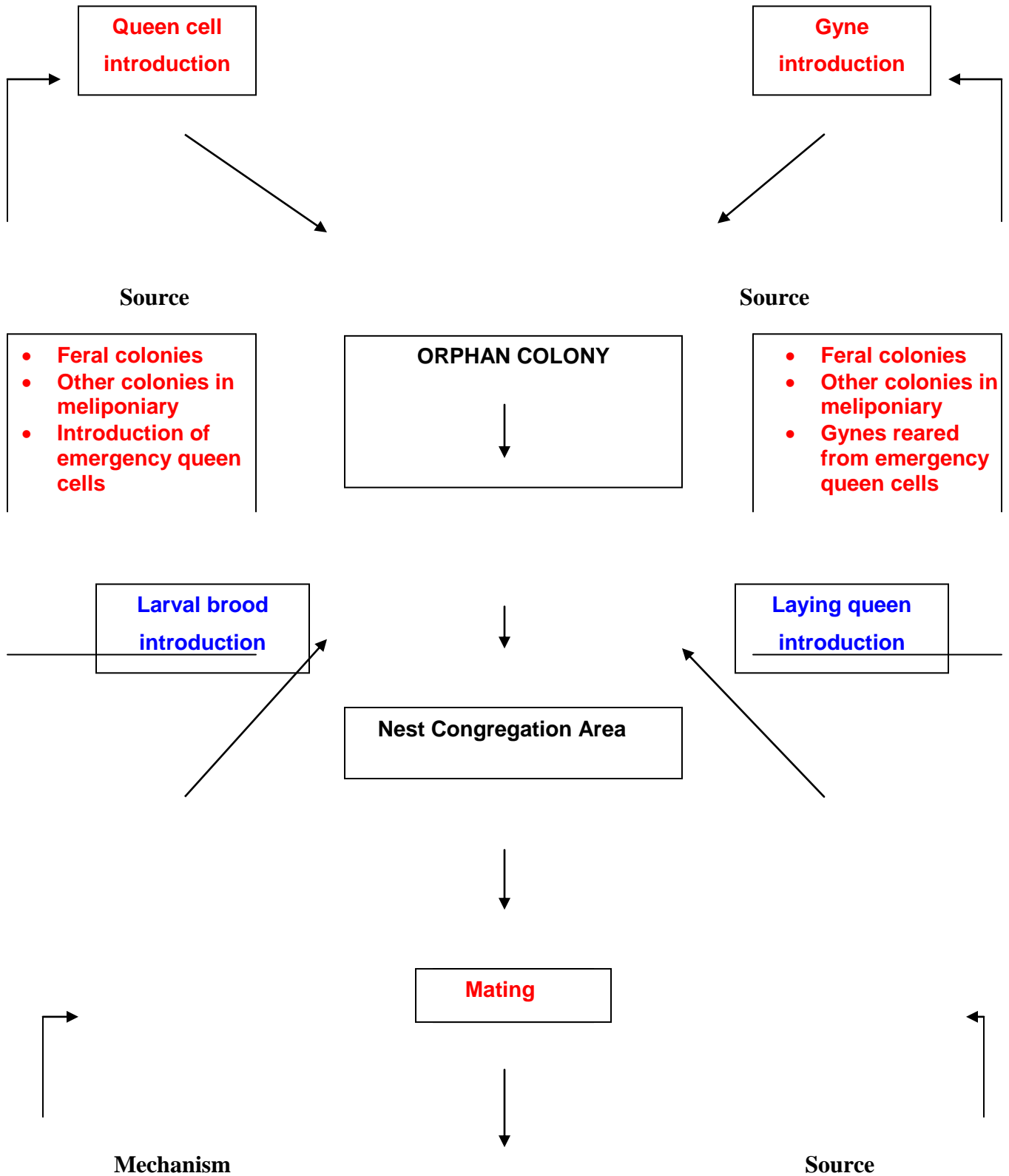
SI. No.	Date of observation	Time of commencement (a.m.)	Time of cessation (p.m.)
1	20.02.'08	7.15	6.34
2	21.02.'08	7.14	6.29
3	22.02.'08	7.14	6.30
4	23.02.'08	7.25	6.33
5	24.02.'08	7.18	6.29
6	25.02.'08	7.20	6.31
7	26.02.'08	7.31	6.28
8	27.02.'08	7.20	6.29
9	28.02.'08	7.55	6.33
10	29.02.'08	7.32	6.28
11	01.03.'08	6.50	6.24
12	02.03.'08	7.03	6.29

Table. 19 Mean time required for resin foraging from different plant sources

SI. No.	Plant source	Gathering and loading time required in (min. & sec.)*		
		Minimum	Maximum	Mean±SE
1	Mango tree (Branch)	2.50	6.18	4.13±0.58
2	Peepul tree (Bark)	4.17	10.11	6.27±0.93
3	Jack (Cracked fruit)	4.49	11.42	6.53±1.09

*Mean of 20 observations

Fig. 10. How to save an orphan colony?



- Worker brood cell
- Auxiliary brood cell
- Link development
- Worker → Queen

Functional Colony

- Weak colonies
- Dequeening
- Queens saved against aggressive swarms



Ripe pupal brood



Strong Colony

Fig. 9. Management of aggressive swarms

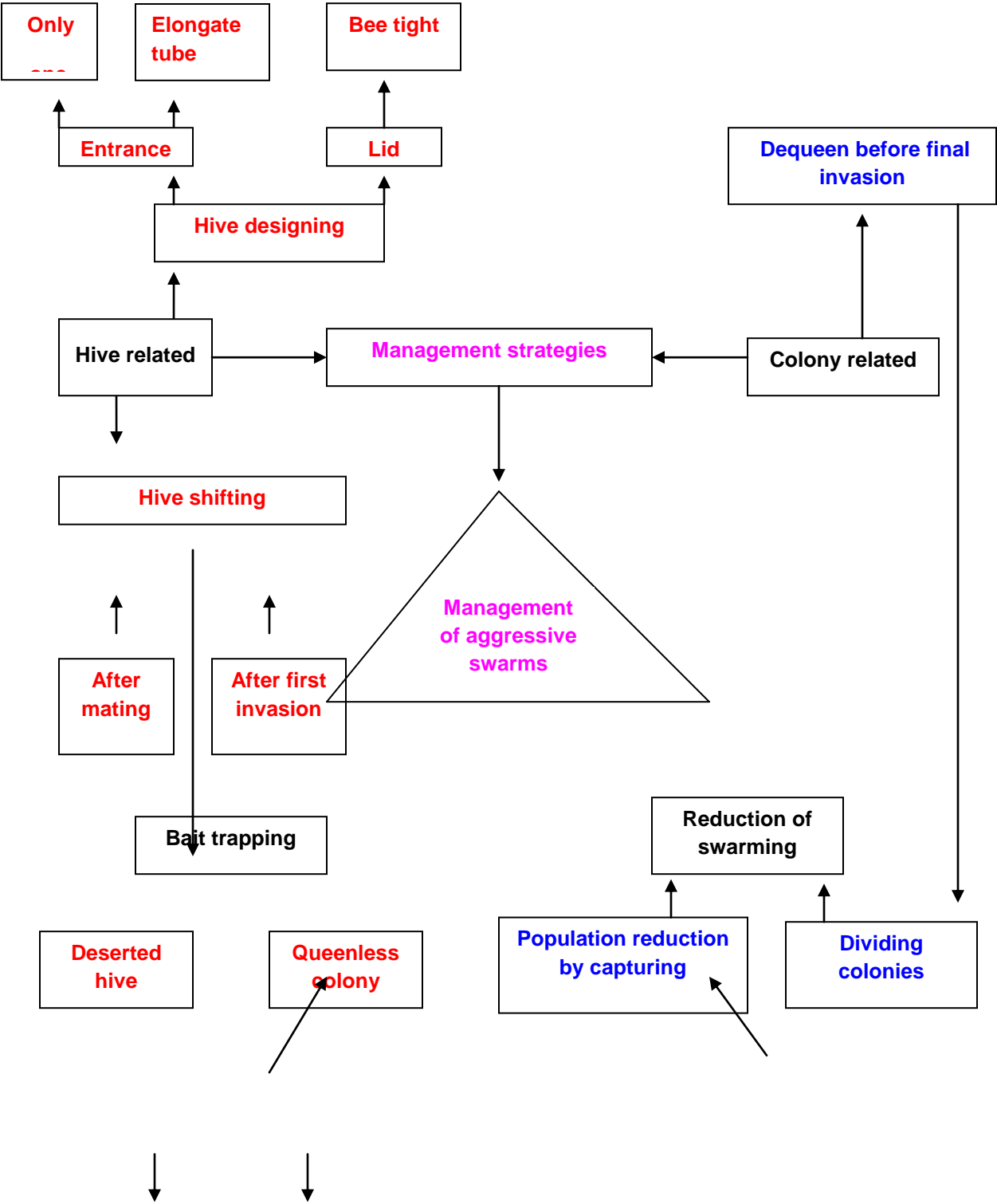


Plate 6. Aonla nursery



Plate 16. Queen cell linked to an auxiliary cell

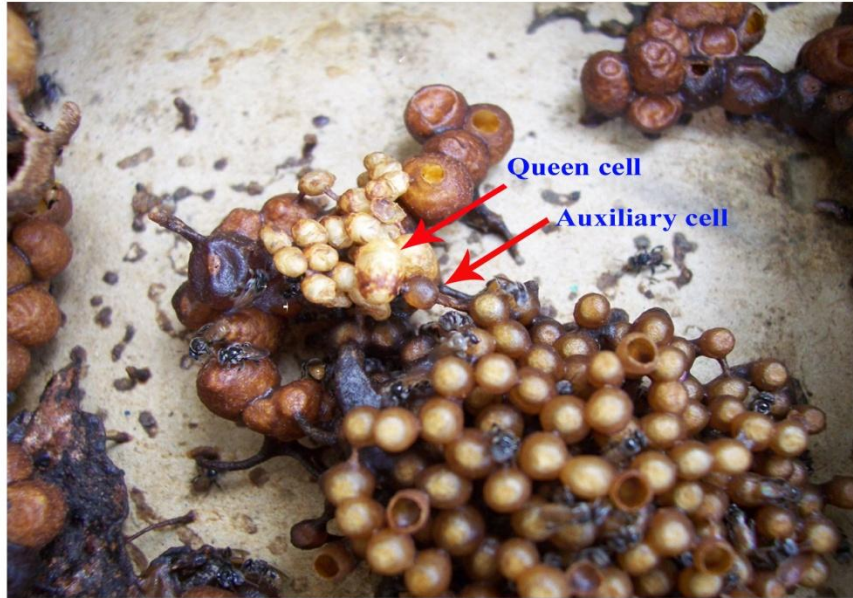


Plate 17. Worker cell linked to an auxiliary cell



Plate 9. Distribution of brood clusters



Plate 10. Attachment of brood clusters

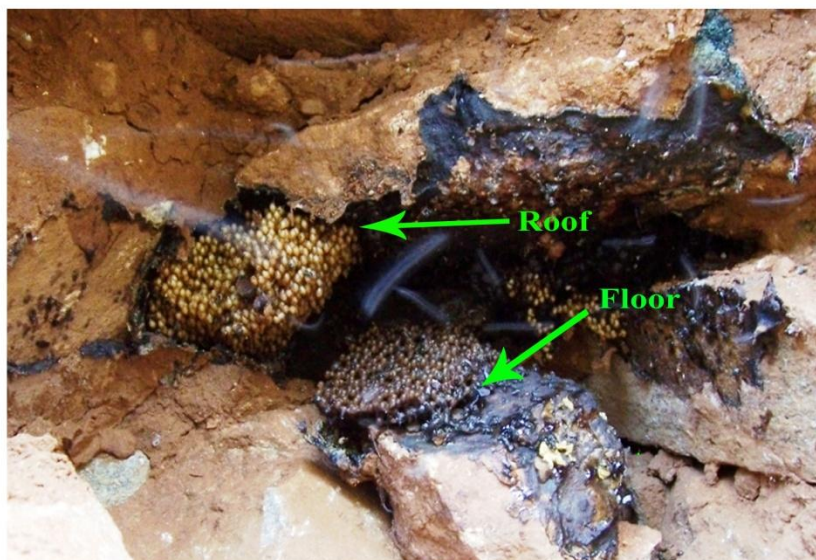


Plate 2. Dilapidated building housing feral colonies



Plate 3. Queen cells



Plate 11. Nest associated drone congregation



Plate 22. Drones resting in a group



Plate 23. Drones resting on a twig

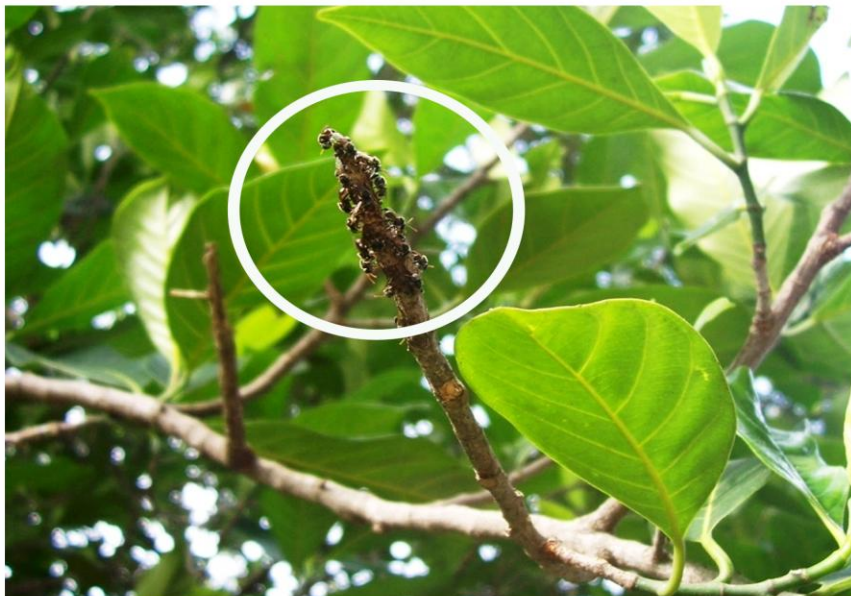


Plate 7. View of an exposed nest

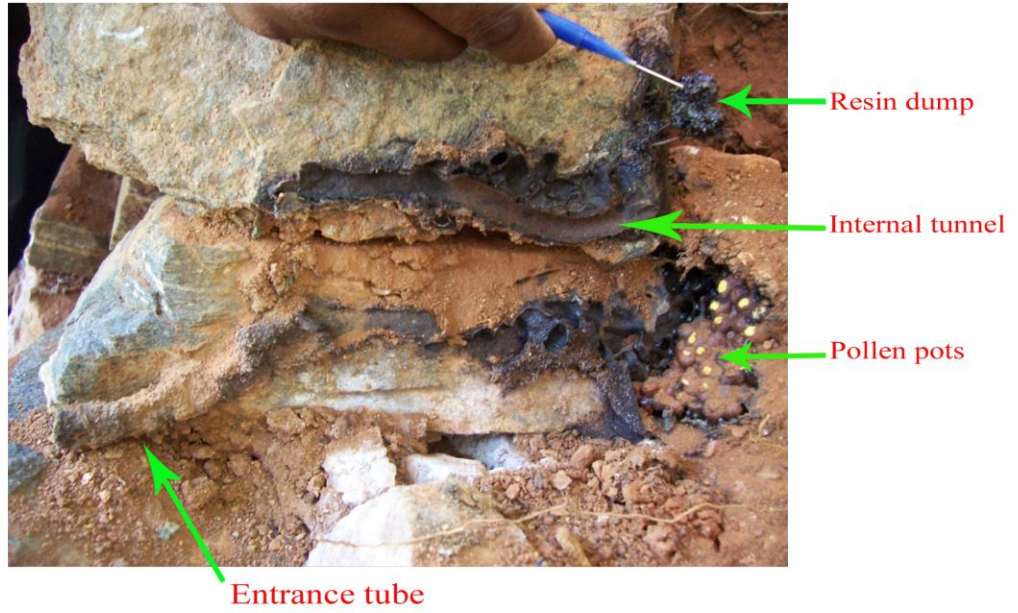


Plate 8. Adjacently placed food pots

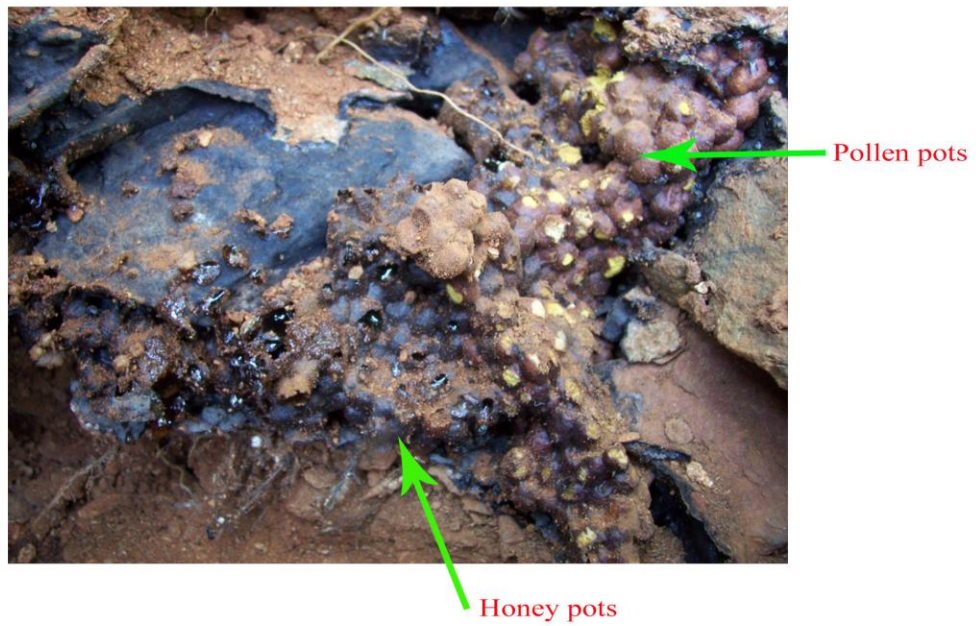


Plate 18. Signs of queenlessness

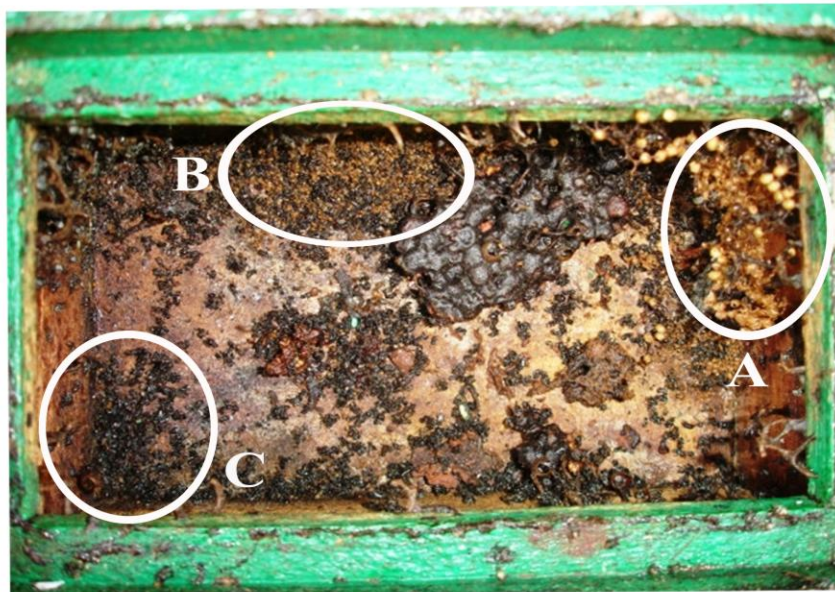


Fake queen cell

Plate 12. Guard bees at the nest entrance



Plate 13. After effects of aggressive swarming



- A- Unremoved empty cocoon
- B- Accumulated waste dump
- C- Dead bees

Plate 4. Gyne



Plate 5. Laying queen



Plate 33. Inflorescence of aonla



M- Male flowers

F- Female flower

Plate 24. An aggressive swarm invading a box hive



Plate 14. Mobbing flight of disturbed bees



Plate 15. Emergency queen cells



Plate 19. Pollen foraging on aonla flowers



Plate 25. Pricking method



Plate 26. Hammer tapping for nest location



Plate 1. Non-nest associated drone congregation



Plate 27. Queen lifting by using a fork



Plate 28. Captured queen inside a plastic container



Plate 20. Resin collection from injured tree bark of mango



Plate 21. Resin flowing out from jack fruit stalk



Plate 29. Resin application around the hive entrance



Plate 30. Refixed entrance tube



Plate 34. Foragers trapped in resin



Plate 35. Intraspecific resin robbing



Plate 31. Safe removal of flightless bees



Plate 32. Hive partitioning with dummy board



