STUDIES ON COLONY REPRODUCTION IN LITTLE BEE

(*Apis florea* Fabricius).

By

S. KALIYAMOORTHY., B.Sc. (Ag)

ID.No.08- 602- 006

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

CENTRE FOR PLANT PROTECTION STUDIES

TAMIL NADU AGRICULTURAL UNIVERSITY

COIMBATORE – 641 003

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Thesis submitted in part fulfillment of the requirements for the award of the degree of **Master of Science (Agriculture) in Entomology** to the Tamil Nadu Agricultural University, Coimbatore - 641 003

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CERTIFICATE

This is to certify that the thesis entitled **"STUDIES ON COLONY REPRODUCTION IN LITTLE BEE (*Apis florea* Fabricius)"** submitted in part fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE (AGRICULTURE) IN ENTOMOLOGY** to the Tamil Nadu Agricultural University, Coimbatore, is a record of bonafide research carried out by **THIRU S. KALIAMOORTHY,** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other 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. N. GANAPATHY**

Date : 13. 05. 2010 **Chairman of the Advisory Committee**

Approved by

Chairman:

Dr. N. GANAPATHY

Members :

Dr. M. MUTHURAMAN

Dr. T. RAGUCHANDER

Date: 30. 06. 2010

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(S. KALIYAMOORTHY)

ABSTRACT

STUDIES ON COLONY REPRODUCTION IN LITTLE BEE

(*Apis florea* Fabricius)

By

S. Kaliyamoorthy

Degree : Master of Science (Agriculture) in Agricultural Entomology

Chairman: Dr. N. Ganapathy
Professor (Agrl. Entomology)
Department of Agrl. Entomology
Tamil Nadu Agricultural University
Coimbatore – 641 003

Colony reproduction in little bee, *Apis florea* Fab. was studied in two experimental florea apiaries established for this purpose with 44 feral colonies hived in specially designed card board box hives using standardized colony hiving and colony shifting techniques during 2009-10.The developmental period varied among colony members. Queen, worker and drone took 15.5, 19.0 and 22.1 days respectively to develop from egg to adult. Colony reproduction occurred mainly through swarming during January-February resulting in the production of two to four daughter colonies from each experimental colony. The primary swarm and the final swarm led by mated queens departed during early afternoon while all other after swarms led by virgin queens departed during late afternoon. Excess queens produced during swarming were eliminated to restore the monogynous status of the colony either in their pupal stage or adult stage mainly by workers. The swarm departure from the natal nest was triggered by the piping sound produced by daughter queen and buzz runs performed by some workers. The swarms usually setteled closer to the natal nest. Queen mating occurred between 12.00 to 13.00 hrs when the drones flight activity was maximum. The mated queen returned to the nest within 15 min after her departure for mating without a mating sign. Workers departed in swarm revisited their deserted mother nest for wax foraging from honey crest and empty queen cells. Colony reproduction also occurred through supersedure and emergency queen rearing. An injured queen was superseded successfully in one colony. A behaviourally different orphan colony could

requeen itself by raising emergency queen cells by modifying the cell bases of workers either from the centre of the comb or along the lower comb edge. In the absence of queen, workers attempted for personal reproduction with limited success in queenless swarms by producing drones where as in queenless colonies worker laid eggs could not develop beyond larval stage. Colony dissolution was the end result since daughter queens raised in all the three methods of queen rearing *viz.*, swarming, supersedure and emergency queen rearing did not inherit the mother nest.

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CHAPTER – I

INTRODUCTION

Honey bees are regarded as superorganisms and they belong to the genus *Apis.* There are three groups of honey bees *viz.,* giant honey bees, dwarf honey bees and cavity nesting honey bees. Dwarf honey bees are the most common honey bees found in tropical Asia. There are two species of dwarf honey bees *viz.,* Red dwarf honey bee, *Apis florea* Fabricius and Black dwarf honey bees, *Apis andreniformis* Smith. *A. florea* is indigenous to Asia. It dwells across the most part of tropical Asia including India, Sri Lanka, Pakistan, China, Thailand and Indonesia and Oman. The workers of *A. florea* are generally reddish brown in appearance and are widely distributed in India. *A. andreniformis* is generally black and its distribution in India is restricted to Assam.

A. florea is regarded as a species of relatively low phylogenic level with in the genus *Apis*. It is an industrious field bee and easy to handle (Ruttner, 1988). It has an enormous Geographical distribution spreading over to nine million square kilometers. It exhibits an invasive potential as it has become established as an exotic bee species in Sudan and Saudi Arabia (Hepburn and Hepburn, 2005). Human predation of little bee colonies is so intense in many areas like Thailand where *A. florea* colonies are widely hunted and consumed. As a result the density of *A. florea* colonies has declined from 200 to 100 per km^2 in Thailand.

A. florea thrives very well in hot, dry climate. It is very well adapted to this environment than other honey bee species. *A. florea* does not require cavities for nesting and are therefore potentially pre adapted to prosper in human altered environments where other species may be constrained by a lack of nest sites.

A. florea builds single comb. It is an open nesting honey bee species often found in the densest thickets. But some nests are so situated that they experience direct sunlight for several hours a day. They use fanning and evaporative cooling to moderate high temperature.

A. florea is not very defensive. A band of sticky resin is deposited around the branch that supports their nests to ward off ants. The bees cannot tolerate even quite minor interference. They adopt a defense strategy of flight instead of fight and desert their nest when the colony is disturbed. This absconding habit mainly deters the attempts to domesticate these bees.

Little bee colonies never stay in the same place for a long time like Bedouin tribes of Oman. Hence these bees are popularly known as 'Bedouin bees' in Oman (Whitcombe, 1984a). Most of the colonies undergo at least one migration per year in search of flora. When it migrates, the flight range of the swarm is not very long.

The annual honey yield from a colony varies from one to three kg. It is believed that little bee honey has medicinal value. This honey has higher dextrin content and has less tendency to granulate than the honey of other *Apis* species. *A. florea* colonies are abundant in the forest and as well as agricultural belts of Kutch region in Gujarat during March-April and in October-November. From this region alone Gujarat State Forest Department gets 100 tonnes of honey annually (Chaudhary *et al.,* 1994). There is an urgent need to upgrade the skill of honey collectors about hygienic method of honey collection without hunting the bees and their brood nest and proper utilization of bees wax, a precious nest product (Soman and Chawda, 1996). Chen *et al.,* (1998) estimated that in Thailand 40, 000 to 50, 000 nests of *A. florea* are sold annually nationwide. The customers buy a complete nest and consume the brood and honey separately. *A. florea* is an excellent pollinator of field and orchard crops. A simple low cost technique of using a 'P' shaped wooden frame for shifting little bee colonies to chosen places would facilitate planned pollination both in orchards and in other agroecosystems (Bhamburkar and Peshkar, 1990).

Densities of *A. florea* can be very high in places where floral resources are rich. Recent bee faunal and floral survey conducted in Tamil Nadu has clearly revealed the scope for keeping florea colonies in places where banana is cultivated extensively and also in tank beds where *Acacia nilotica* and *A. planifrons* are grown. The nest densities of *A. florea* are also found to be high in energy plantations established in Ramanathapuram, Sivagangai and Tirunelveli Districts of Tamil Nadu with *Prosopis juliflora*. This tree is usually well preferred by the bees for their nesting. In addition, it is also a major source of nectar and pollen for the bees.

A. florea has received little scientific attention, even though this species has been described more than two centuries ago by Fabricius in the year 1787. The knowledge about *A. florea* is fragmentary and most are assumptions extrapolated from discoveries about *A. mellifera.* Colony reproduction of *A. florea* is not well studied. Similarly, the mating biology of this bee is not yet thoroughly understood as the places of occurrence of drone congregation areas are still awaiting discovery. In addition to reproductive swarming *A. florea* also undergoes migratory swarming in which the colony abandons its nest and moves in search of more profitable pastures. Detailed studies on swarming behaviour will help us understand colony reproduction which in turn will be useful to evolve colony management strategies.

A. florea can be partially domesticated. The feral colonies of *A. florea* can be cut and shifted to shady places both for pollination and honey production. The attempts made so far to design a hive for keeping *A. florea* colonies have not met with total success. Designing a low cost hive suited both for colony transport and colony maintenance is essential for establishing an experimental florea apiary where several observations about bee behaviour can be studied conveniently.

To bridge some of the important research gaps the present investigation was taken up with the following objectives,

1. Establishing an experimental *A. florea* apiary
2. Investigating the developmental biology of colony members
3. Understanding the colony reproduction that occurs in the presence as well as the absence of queen

CHAPTER – II

REVIEW OF LITERATURE

The dwarf honey bees, *A. andreniformis* and *A. florea* are two distinct species. They are almost the same size; both build a single exposed comb and may utilize similar resources in the same or similar habitats. However, they are reproductively isolated. Reproduction in honey bee colonies can be partitioned into two distinct categories *viz.,* individual and colony reproduction. Reproductive strategies of the colony vary under queenright and queenless situations. Literature pertaining to colony reproduction in dwarf bees with special reference to *A. florea* is reviewed here.

2.1 Colony reproduction in queen right colonies

Sex determination in honeybees occurs through haplo-diploidy mechanism. The male sex results from unfertilized eggs and the female sex originates from fertilized eggs. A very conspicuous characterstic of *A.florea* is the striking difference in size between worker bees and sexual forms. The inequality is much smaller in other *Apis* species. Differences in body sizes are reflected also in the diameter of brood cells. Before oviposition the queen determines the size of the brood cell with her front legs. Whenever she encounters cell of a wide diameter (drone cell) she deposits an unfertilized egg. If it is a smaller cell then she will lay fertilized egg. Obviously the cell size is an important factor for sex determination. It is solely the workers that construct combs and they limit drone cell construction. Therefore the workers have considerable influence on the maximum number of drones possibly produced in a colony. Nutritional status of the colony also affects the regulation of drone production. The arrhenotokous parthenogenetically produced haploid male sexual reproductives (drone) are often misnamed as a caste. The female sex of the honey bee alone exhibits functional polymorphism. Hence, queen and worker alone are the true castes in honey bee colonies. Drones obviously represent the male sex but not a specific caste. Like queen the workers are also capable of laying drone producing haploid eggs through individual reproduction normally in queenless colonies (Moritz and Southwick, 1992).

Developmental biology of *A.florea* was studied in detail by Sandhu and Singh (1960). The developmental durations of different colony members are given below.

Developmental duration of life sages of different colony members of *A.florea*

Colony member	Duration in days			
	Egg	Larva	Pupa	Total developmental period
Queen	3	6.8	7.7	17.5
Drone	3	6.7	12.8	22.5
Worker	3	6.3	11.2	20.5

Swarming

Swarming represents the main reproductive phase in the life cycle of the honey bee colony. Swarming in *A.florea* may announce the approaching end of an individual colony, a trend which does not appear so clearly in the other *Apis* species. *A.florea* colony usually begins its growth trajectory from a cluster of broodless, combless adult bees. The worker bees construct combs, nurse brood and store honey. Finally when a matured stage is reached with most of the brood sealed, they construct queen cells at the lower edge of the comb. Now the bees stop expanding the nest and rearing new brood. Immediate swarming preparations are marked by general restlessness and repeated runs of workers and the queen on the surface of the bee curtain. Now every few days another swarm issues as young bees and queens hatch out successively (Lindauer, 1956; Tirgari, 1971).

Behaviour of virgin queens

The first virgin queen comes out shortly before or after the first swarm leaves, probably together with the mother queen (Tirgari, 1971; Whitcombe, 1984b). The newly emerged queen of *A. florea* patrolled mainly on the comb beneath the curtain of bees which covered the colony. On third and fourth day after emergence, the queen crosses the honey crest several times. Virgin queens of *A. andreniformis* often produce piping rounds with rapid tempo. Queen piping sounds induce a cessation of activities in *A. andreniformis* workers. Removal of the piping queen from the nest of *A. florea* caused the emergence of several virgin queens. One remained on the comb and was chasing the others away for several days (Wongsiri *et al.,* 1996). Queen–queen conflict was first observed by Koeniger. He found this conflict in one colony where one virgin queen emerged when another virgin queen was still in the colony. A fight ensued and only one queen survived finally. None of the sealed queen cells were damaged by virgin queen. However, Wongsiri *et al.,* (1996) reported that daughter queens co exist together without any rivalary.

Swarm departure

While first swarm takes with it about half of the colony, the following swarms become smaller and smaller until a little group of bees is left on the big comb which leaves with the last queen or disperses. *A. florea* workers return to old nest to collect the wax from old comb. Only a few bees come to collect the wax from the nest and from old queen cells.

Colonies of *A. florea* are almost as peripatetic as those of giant bees with most nests undergoing at least one migration per year (Akaratanakul, 1977; Wongsiri *et al.,* 1996). *A. florea* often undergoes migratory swarming in which the colony abandons its nest and moves on in search of profitable floral resources resulting in the complete dissolution of the colony. The last surviving daughter queen in a strong colony may inherit the mother nest very rarely and a second swarming cycle may start later (Whitcombe, 1984b).

The tropical bees form too many swarms. The chances of survival of small swarms under poor conditions become less. There is a point obviously at which no more swarm should be produced because they become too small to reach even maturity. The number of swarms issued from an individual colony varies. Lindauer (1956) observed five swarms from an individual colony in Sri Lanka within a period of 12 days.

Nesting sites

Scout bees play a crucial role in nest site selection by swarms in *A. florea*. They perform both directional and nondirectional dances to indicate the nest site location to the workers participating in the swarm. These dances are performed in the mother nest prior to swarm departure (Oldroyd *et al.,* 2008). *A. florea* reproductive swarms do not form an interim cluster. They move directly to their new nesting site which is selected via competitive dancing before swarm take off. However, they may use a temporary cluster when they abscond from the nest after disturbance (Oldroyd and Wongsiri, 2006). The distance of a new nesting site of a swarm may be only a few up to several hundred meters (Tirgari, 1971). For example a reproductive swarm of *A. florea* settled about 20 m from its natal colony in a short tamarind tree (Akaratnakul, 1977).

Mating biology

Sexually mature drones fly off in large numbers on warm, sunny days and aggregate as drone assembly in drone congregation areas to mate with queen . The timings of mating flights of *A. andreniformis* and *A. florea* are well separated temporally. *A. andreniformis* fly just after the sun passes its zenith and *A. florea* fly later in the afternoon. Full mating flights of 15 to 30 min duration occur when queens are six to eight days old. The first mating flight of *A. florea* was between 14.00 and 15.00 hrs (Wongsiri *et al.*, 1990).

Mating of queens with drones is a spectacular event. Queen mates with several drones in succession. Drones die immediately after mating. In dwarf bees the semen is directly injected into the spermatheca via a specially designed endophallus (Koeniger, 1990). Hence, there is no wastage of sperms. The successful but yet dead drone leaves a part of the endophallus within vaginal duct of the queen which does not protrude outside. Hence, *A. florea* drones do not leave any mating sign. (Koeniger *et al.*, 2001)

2.2. Colony reproduction in queenless colonies

A. florea is highly polyandrous and so most workers are related as half sisters. Based on kin selection theory an individual worker can gain greater fitness from personal reproduction (relatedness to sons = 0.5) than by raising sons of either queen (relatedness to queens sons = 0.25) or sons of half sister workers (relatedness to the sons of half sisters = 0.125) (Ratnieks, 1988). Reproduction by worker is therefore not in the genetic interest of other workers. Thus a conflict exists between the reproductive interests of individuals and that of collective workers. This conflict can be resolved among workers by delegating production of male eggs to the queen (Frank, 1995).

Worker reproduction in *A. florea* is curtailed by policing mechanism that keeps worker reproduction at an extremely low level. Any behaviour of worker that lowers direct reproduction by other workers is referred as worker policing. An important component of worker policing behaviour is oophagy of worker laid eggs. Queen of *A. mellifera* and *A. florea* mark their eggs with a pheromone. Worker lacks this pheromone and other workers therefore eat their eggs (Ratnieks, 1995). Under these circumstances workers are expected to evolve functional sterility in the presence of queen.

Reproductive behaviours of *A. florea* workers are complex and variable. Workers with activated ovaries are very rare in queen right *A. florea* colonies (Halling *et al.,* 2001). Workers activate their ovaries only a few days after queenlessness (Nanork *et al.,* 2005). Worker oviposition occurs soon after the worker ovary activation. Despite functional sterility when colonies are queen right, when a colony become queenless activation of ovaries occur in many young workers. They lay eggs which may produce a final batch of males before the colony perishes (Halling *et al.,* 2001; Oldroyd *et al.,* 2001). To rear the worker laid eggs the colony must undergo a fundamental behavioural shift and cease worker policing for without successful reproduction the fitness of a queenless colony is zero.

Laying workers are more active in hopelessly queenless colonies. They disappeared after the emergence of virgin queen. The eggs laid by laying workers were longer, wider and thicker than queen laid eggs (Woyke and Wongsiri, 1992). A worker in a hopelessly queenless colony has three alternative reproduction strategies (Nanork *et al.,* 2006). First she can remain in the natal colony and raise her own offspring and those of her sisters. Second a queenless worker can abandon her own colony and find another queenless colony to parasitize that colony with her eggs. Third she could join a queen right colony. A worker that remains in her own colony may have reduced reproductive success because some of the colony's resources are utilized to produce offspring of nonnatal parasites. Further, queenless colonies almost always abscond before successfully raising mature drones. Queenless workers that move into a queenright colony face a reproductive dead end as any egg she lays will be policed by other workers and she will invest in the rearing of offsprings to which she is unrelated. Thus for some workers, joining another queenless colony and becoming a parasite rather than being parasitized is the best option. This option gives more chances for individual reproduction and increases the fitness of the entire queenless colony. Recent studies have revealed the existence of such inter colony parasitism or worker reproductive parasitism in *A.florea* (Nanork *et al.,* 2005, 2006).

In queenright colonies non natal workers are present at low frequency of two percent (Nanork *et al.*, 2005). However, after loss of queen the proportion of non natal workers increases to 4.5 percent. The proportion of nonnatal workers increases significantly when colonies become queenless and these workers are more likely to have active ovaries and lay more eggs than natal workers. As a result queenless colonies are heavily parasitized with the eggs of nonnatal workers which is called inter colony parasitism or worker reproductive parasitism.

CHAPTER - III
MATERIALS AND METHODS

The present study has been taken up mainly to understand and gather more information about colony reproduction of *A. florea* during 2009 to 2010. The materials utilized and methodologies followed in the present study are described below in detail.

3.1. Establishment of *florea* apiaries

Two experimental *A. florea* apiaries were established to carry out these studies with 44 field collected colonies to study colony reproduction in *A. florea*. The feral colonies were hived and maintained in specially designed hives. The florea apiaries were set up at two locations *viz.*, Ganapathy and Chinnavedampatti, ten kilometer away from Coimbatore (Plate 1). A low cost, card board box hive was specially designed for the purpose of shifting the colonies from field to the experimental apiaries. The feral colonies were successfully maintained in these hives throughout the study period.

3.1.1. Card board box hive

A simple card board box hive was designed (Fig. 1). The hive was very well suited both for the purpose of transportation and maintenance of feral colonies at two out apiaries. The dimensions of the card board box hive were fixed according to the size of the feral colony. Two 'U' shaped cuts were made on each side along the side walls of the card board box which served as rabbets. The width of the cuts was fixed according to the thickness of the twig to which the comb was attached. Similarly the depth of the cuts was fixed according to height of the honey crest so as to accommodate the honey crest conveniently within the hive even after closing the card board box using the top lid. Small holes of two mm dia were made on the sides and the front wall of card board box for ventilation. A rectangular opening was cut on the front wall of the hive along the perforations to facilitate the landing and take off by foraging bees from their nest after shifting the colonies to florea apiary (Plate 2).

3.1.2. Hiving of feral colonies

Arboreal nesting colonies were mainly hived. The shrubs and trees from which the colonies were hived are given in Table 1 and 2.The twig bearing the comb was gently cut by using either a secateur or a small hack-saw blade with least disturbance to the comb and bees. Most of the bees which flew out while cutting the twig hovered in air or settled near their nesting sites. The twig with the comb thus removed was kept inside the card board box hive in the 'U'shaped rabbets. Later the card board box hive with the comb was kept closer to the original nesting site with top lid open. All the bees settled on the comb within 15-30 min. Then top lid of the hive was closed. All the edges of the hive were sealed by using gum tape prior to transport and the hive was made bee proof.

3.1.3. Transport of hived colonies

The feral colonies of *A. florea* thus hived in card board box hives were transported to experimental apiaries by using either a two wheeler or a four wheeler after dusk. Colony removal, hiving and shifting were mainly done during the evening time to avoid the loss of field bees. Care was taken to minimize jerks during transport to avoid breakage of combs. The front lid on the front wall of the hive was removed after reaching the apiary site.

3.2. Developmental biology

Biology of queen, worker and drone of *A. florea* were studied in three experimental colonies maintained at florea apiary during January - February 2010. Ten empty worker cells in two rows of five each were selected nearer to the periphery of worker brood area. The row of selected cells was marked by inserting two pins at the beginning and end of the selected row (Plate 3). The brood cell marking methodology followed by Sandhu and Singh (1960) was slightly modified and followed. Instead of marking the cells individually a line of five cells was marked. The similar method of cell selection and marking procedure were followed for drones in the drone brood area. The developmental biology of queen was studied both in swarm cells and also in emergency queen cells. The queen development was continuously monitored from the stage of queen cup construction until emergence. The relative position of the selected queen cells for the study was fixed and numbered by using a rough sketch of queen cells. Progressive changes that occurred in the queen cells were continuously observed. In addition, cursory observations were also recorded to find out the pre pupal period of drone, worker and queen. The cappings of selected sealed brood cells were removed daily and the life stage found inside was observed until pre pupa metamorphosed into pupa.

The developmental duration of different life stages were worked out for all the three colony members as follows.

a) Egg period	:	Time gap between egg laying and hatching.
b) Larval period	:	Time gap between hatching of larva and sealing of brood cell.
c) Pre pupal period	:	Time gap between sealing of brood cell and larval pupal moulting.
d) Pupal period	:	Time gap between sealing of brood cell and adult emergence

3.3. Colony reproduction in queen right colonies

3.3.1. Swarming

The swarming behaviour of *A. florea* was studied during the peak swarming season *i.e,* January to February 2010 in five colonies maintained in the florea apiary at Ganapathy. Cursory observations were also recorded in other colonies which were also in swarming phase. The various swarming events starting from drone rearing up to the dissolution of the colony were studied by making *in situ* observations daily from dawn to dusk. The prolonged observations were made possible with the assistance of an experienced beekeeper in addition to the researcher.

Sequential progress of various preparatory events connected with swarming like drone rearing and queen rearing were studied by monitoring the changes that occurred in the brood nest. The number of daughter queens reared successfully in each colony was recorded by distinguishing and counting the various stages of queen cells, normally sealed queen cells, open empty cells and damaged cells. Chronological data pertaining to the occurrence of primary swarm, after swarms and final swarm were recorded. The number of swarms produced per colony was also counted. The behaviour of the daughter queens on the natal nest was visually monitored and also video documented. Similarly, the antagonistic behaviour of worker bees towards the virgin queens and their communication behaviour just prior to the swarm departure were recorded. The mechanism of virgin queen elimination before their emergence was found out by observing the fate of sealed queen cells. Likewise, the cause of queen elimination after their emergence was found out by recording the occurrence of queen – queen conflict or/and queen – worker conflict either by constant visual observations and/or video recording. Thus various queen elimination mechanisms were found out and their percent contributions worked out. The wax foraging behaviour of the departed workers from the deserted mother nest was also recorded.

3.3.2. Supersedure

Queen production mechanism by supersedure was studied in two colonies.

Colony 1: A feral colony nesting on a wire fence, making efforts to replace its queen was hived and brought of the florea apiary. The colony was queenright. There was a sealed queen cell at the bottom of the comb. The worker brood cells were under varying stages of development constituted the brood nest. The progress and the final outcome of supersedure were closely monitored to understand the colony reproduction under supersedure.

Colony 2: A feral colony in which the drone brood rearing was just started was hived and brought to the florea apiary. Later the colony extended its efforts for swarming by building several queen cells. The wings of the queen were cut diagonally to prevent the departure of mother queen with the primary swarm. The queen replacement which occurred in the colony due to the injury inflicted to the queen was studied.

3.3.3. Mating flights

3.3.3.1. Drone mating flight

Mating flight timings of drones were studied in three chosen colonies which had many drones of unknown age. The study was conducted during swarming season in the month of February for a period of ten days. The drone mating flight activity was studied from 11.00 to 16.00 hrs at thirty minutes interval. The total number of drones departing from each colony over a period of five minutes was counted. In addition, the time of the earliest drone flight, time of final afternoon return flight and peak period of drone flight activity were recorded.

3.3.3.2. Queen mating flight

The queen mating flight behaviour was studied in one superseded queen. The original queen of that colony which was preparing for swarming was made flightless by making one longitudinal cut across each of her fore wing. The queen was superseded because of the injury inflicted. The queen mating flight behaviour of the superseding queen was studied. The time of departure for mating, the time of arrival after mating and the mating flight duration were recorded in the colony by observing the take off and landing time of the queen from the inherited nest. The mated queen was checked for the presence of any mating sign at the tip of her abdomen. In addition, the fate of the drones in that nest after mating was studied and video documented. The antagonistic behaviour shown by the workers against drones was also recorded.

3.4. Colony reproduction in queenless colonies

Colony reproduction was studied in three colonies. Colonies became queenless either due to loss of queen during capturing or worker aggression. Efforts of the colony to restore queenright status were recorded. In addition, the impact of queenlessness on the bee behaviour and colony defense behavior was also studied. Defense behavior of the colony was assessed by observing the selective changes that occurred in the structure of the bee curtain. The aggressiveness of the bees was found out by their inclination to sting the intruder. Intensity of bee traffic was estimated by counting the number of foragers landing per minute. Bee count was taken at 30 minutes interval during the peak foraging time ie, 10.00 to 12.30 hrs. Various events associated with requeening process *viz.,* origin and fate of new queens produced and worker egg laying commencing from the construction of emergency queen cells upto the desertion of the colony were recorded.

Colony reproduction status was also studied in a hopelessly queenless colony. This condition was created by pinching all the emergency queen cells built by the queenless colony. Cursory observations were also recorded in that colony pertaining to worker egg laying and fate of worker laid eggs and the colony. In addition, the reproductive behavior was studied in three queenless swarms for their perpetuations were studied. Cursory observations related to the character of swarm cluster, comb building and drone development were also recorded

3.5. Data analysis

The data on developmental biology were statistically analyzed by using CRD design. The means were compared by following LSD procedure. The AGRES software package was used to carry out the analysis.

CHAPTER - IV

EXPERIMENTAL RESULTS

4.1. Establishment of florea apiary

Establishing a florea apiary was aimed to bring feral colonies of *A. florea* at one site so that various studies could be taken up easily. Conducting experiments with feral colonies distributed at different locations will be a difficult task. This difficulty was mainly solved by establishing florea apiaries. Two florea apiaries were successfully established with 44 field collected colonies at two locations (Plate 1). The card board box hive was found to be best suited for transport and colony maintenance. Out of 44 colonies hived 15 colonies successfully stayed in the apiary for nearly three months even after the completion of the study.

4.2. Developmental biology

The data pertaining to the developmental biology of various castes of *A. florea* are presented in Table 3. In all the three colony members, the eggs hatched into larvae within three days. The larval and pupal durations varied significantly among different colony members. Larval development was faster than pupal development. Development of eggs and larvae occurred in open cells. Both prepupal and pupal stages were found inside the sealed cells. The developmental durations in the open cells and closed cells were almost equal in all the three colony members (Fig. 2). The total developmental duration of queen, worker and drone were 15.6, 19.0 and 22.1 days respectively. Queen developed much faster than worker and drones. The drones took the maximum time for their development (Table 3). Both workers and drones developed in hexagonal cells whereas the queen grew inside the vertically suspended, thimble shaped cells (Fig. 2).The freshly emerged drones were fed by workers.

4.3. Colony reproduction

4.3. 1.Swarming

The beginning of drone brood rearing marked the commencement of swarming in four out of five colonies studied. During swarming season swarm cells were mainly built along the lower edge of the comb often in small clusters (Plate 4).

The number of swarm cells built ranged from three to eleven. Most of the queen cups built were elaborated into queen cells. Queens emerged from half of the sealed cells (Table 4). The workers removed wax from the matured queen cell two to three days prior to the emergence of the queen (Plate 5). A circular lid found at the tip of the empty queen cell indicated the satisfactory emergence of queen from sealed queen cell. Such empty cells after queen emergence were again sealed often perfectly by workers. Trapped workers were seen inside such cells with resealed caps rarely. Nearly 50 per cent of the sealed cells were damaged at the side with the tip still intact for destroying the developmental stage of the queen dwelling inside, either by queen or workers.

The various swarming events observed in the experimental colonies just prior to the swarm departure until settling are described below. Both the queen and workers became restless just prior to the onset of the swarm issue. The daughter queen was found running aimlessly on the bee curtain. She was found to produce an audible piping sound for a few seconds at frequent intervals. Many workers and drones were found on the honey crest. They sipped and filled their honey stomach with honey. A few worker bees on the bee curtain exhibited dorso-ventral abdominal vibrations. A few other worker bees were found running in straight lines on the bee curtain in an irregular fashion. Such bees intentionally established contact with other bees and recruited them for performing buzzing runs. These runs gradually intensified leading to the take off of a part of worker bees resulting in the formation of a swarm.

The queen was the last member to join the swarm. Workers returned to the mother nest either once or twice whenever the queen failed to join the swarm. The swarm usually settled closer to the mother nest and established a new daughter colony. The bees in the swarm cluster started their comb building activity a day after their arrival. Egg laying by the queen started two days after the comb construction. The swarms subjected to disturbance absconded from their new nesting sites. The workers of the absconded swarm were also found to collect wax from the newly built nest both from honey crest and along the outer margins of the comb. The number of after swarms produced per colony varied from two to four (Fig. 3).

The peak swarming season was found to be from January to February. The primary swarm was always led by the old queen. Departure of primary swarms occurred in the evening between 13.20 to 14.15 hrs (Fig. 4). In general the emergence of first daughter queen occurred two to three days after departure of the primary swarm. The first emerging daughter queen always led the secondary swarm. Daughter queen led after swarms departed between 15.12 to 16.30 hrs in the evening just prior to the emergence of next daughter queen. The emergence of second daughter queen occurred within one and half hour after the departure of secondary swarm. This queen either led the tertiary swarm or final swarm deserting the mother nest leading to the dissolution of the colony.

The last emerging daughter queen nibbled the queen cells which paved the way for elimination of other developing queens inside the queen cells prior to her departure. Workers played a crucial role in extending the damage done to the queen cells by the queen. They were mainly responsible for pre emergence destruction of queens inside the queen cells. The dead queen found inside the damaged cells were subsequently removed by the workers.

Daughter queens also emerged one after another in two experimental colonies (Table 5). The two queens stayed together without any conflict in colony 4. No queen duel was observed. However, one of the queens was killed by an unknown mechanism. The surviving queen led the after swarm. Similar kind of emergence of daughter queens was also found twice in colony 1 (Table 5). During the first occasion one of the queens was killed due to the balling by the workers on the mother nest itself. During the second occasion both the daughter queens led the tertiary swarm. The excess queen elimination occurred in the swarm cluster by an unknown mechanism.

Dissolution of the colonies occurred after departure of the last swarm in four out of five colonies. The final swarm led by the last surviving daughter queen usually completed its mating prior to departure. She left the mother nest with remaining workers. She laid eggs in the cells prior to departure. However, the departed workers revisited the mother nest for taking away the wax especially from the honey crest. They also rarely collected wax from the base of the empty queen cells (Plate 6). In such departed nest the wax moth infestation became more common leading to the destruction of the comb by their larvae (Plate 7). However, colony dissolution did not occur in colony 4. The last surviving daughter queen did not depart from the mother nest. She instead inherited the mother nest as in cavity nesting bees (Table 5).

4.3.2. Supersedure

The study conducted with colony 1 indicated the possibility of queen replacement whenever a queen was injured. In that colony the swarming process ultimately ended in supersedure with the daughter queen inheriting the mother nest after replacing the injured queen. In this colony the excess queen cells were torn down. The drones were also evicted from the colony after the safe arrival of the superseded queen from her successful mating flight. Workers exhibited hostility towards the drones. A group of four to five workers clinged to the drone and jointly staged an attack against each drone. The workers pulled and chewed the wings and legs of the drone. The drones subjected to such attack fell off on bottom floor of the experimental hive. The injury inflicted to the drones rendered them flightless. The workers continued their attack against the fallen drones even on bottom floor of the experimental hive (Plate 8)

In colony 2 the daughter queen successfully emerged out of the sealed queen cell and deserted the nest. The queen replacement occurred in both the experimental colonies used for studying supersedure after the elimination of the mother queens by some unknown elimination mechanisms.

4.3.3. Mating flights

4.3.3.1. Drone mating flight

Drones appeared on the bee curtain and walked upward. Subsequently they flew out of the nest**.** The earliest drone flight started only at 11.00 hrs in the experimental colonies. Mating flight timings of drones of *A. florea* are presented in Fig. 5. The peak period of drone's mating flight activity occurred between 12.00 and 13.00 hrs. The cessation of flight of drones occurred at 15.30 hrs.

4.3.3.2. Queen mating flight

Queen mating flight was observed in a colony which was preparing for swarming but ended in supersedure. The superseded queen left the inherited nest at

12.16 hr for mating at the age of eight days. The queen reentered the nest at 12.32 hr after mating. No mating plug was noticed at the anal end of the mated queen.

4.4. Colony reproduction in queenless colonies

Studies conducted to understand the reproductive dilemma of queenless colonies revealed many interesting results (Table 6). Queenlessness markedly altered the defense and foraging behaviour of worker bees which greatly reduced the sustained survival of these colonies. Worker bees in queenless colonies were highly aggressive against intruders and alien bees. The bee traffic was very much affected. There was marked decline in the number of incoming foragers (Table 6). The protective bee curtain became less compact. The bees on the bee curtain became disorganized. The bees were found to hang in loose clusters (Plate 9). Cluster forming behavior was markedly affected in queenless absconding swarms. The bees spread out on a branch of a tree and remained scattered without forming a swarm cluster (Plate 10).

However, all queenless colonies made efforts for requeening by constructing emergency queen cells. The site and time of construction of these queen cells varied. Out of three colonies studied, in two colonies the queen cells were built by modifying the lower most worker cells found along the lower edge of the comb and in one colony the emergency queen cells originated from the central zone of the worker brood area (Plate 11). In two colonies the queen cell construction commenced on the very next day after queen loss and in one colony after the lapse of five days (Table 7). The queen cell base was constructed by modifying a few worker cells. Worker larvae were mainly used for emergency queen production. The number of queen cells built also varied from two to thirteen (Table 6). Queen emergence was noticed nine to eleven days after the appearance of queen cells (Table 7). After the emergence of the first queen, the other sealed queen cells were destroyed mainly by workers though the first emerging queen initiated the queen cell attack by nibbling. The newly emerged queen departed with all workers in colonies which were in growing phase which led to the dissolution of the colony. However, in colonies which were making preparations for swarming the first emerging queen produced out of emergency impulse departed with a part of the worker population before the emergence of the other queens. The destruction of queen cells were delayed until the emergence of second queen which also left the colony along with the remaining workers leading to the dissolution of the colony.

The absence of queen in queenless colonies was found to trigger worker egg laying. Workers glued few eggs to the side walls of the worker cells (Plate 12). Though many eggs were laid by the workers only a few eggs hatched out as larvae due to effective worker policing. None of them were able to reach the pupal stage. A cursory observation made in a hopelessly queenless colony revealed the existence of intense worker egg laying activity in the absence of queen. A maximum of 13 eggs per cell was recorded in that colony (Plate 12). Similarly in another orphan colony hundreds of eggs were found scattered on the floor of the experimental hive (Plate 13).

The queenless swarm was also inclined to build the comb. But the rate of comb building activity did not progress as observed in queen right swarm. The bees in the swarm built only drone cells in the brood nest. They also built honey crest above the twig and stored the honey. The drone cells contained two to four worker laid eggs per cell (Plate 14). However, from each cell one drone alone developed successfully at the end. The bees were found to store honey not only in the honey crest but also in the brood region (Plate 15). The results obtained clearly revealed that queenlessness when occurred in any colony or a swarm always led to the dissolution of the colony due to absconding.

CHAPTER – V

DISCUSSION

The results obtained in present study carried out on the colony reproduction in *Apis florea* are discussed here under various heads in detail with appropriate supporting evidences.

5.1. Establishment of florea apiary

Establishment of florea apiary is an essential prerequisite for any kind of experimentation. In order to achieve this goal sustained efforts were made to hive florea colonies from various locations. During the study period 44 *A. florea* colonies were hived in specially designed card board box hives. However, only 15 colonies successfully remained in the hives for more than 100 days and the remaining colonies deserted after staying for a period of 31 to 85 days (Table 2). Though there were difficulties initially in hiving and transporting the feral colonies, they were overcome by making suitable corrective measures. The experience gained during hiving process and subsequent transport of hived colonies to the study site was useful to evolve strategies for successful hiving of *A. florea* colonies which are listed below.

Dos

- ❖ Hive the nest either during early morning or late evening.
- ❖ Choose the appropriate cutting tool according to the size of the twig. A small twig is easily cut with secateurs while a hack saw blade is suited for cutting a large twig.
- ❖ Delay the shifting of nest until all the bees settled back on the comb.
- ❖ Transport the hived colonies with minimum jerk.

Don’ts

- ❖ Do not hive the colonies with comb white or yellow.
- ❖ Do not hive the colonies which are queenless.
- ❖ Do not hive the colonies which are ready to swarm.
- ❖ Do not catch the queen with fingers as handling queen may inflict injury to her.

- ❖ Do not expose the naked comb to direct sunlight to avoid melting of combs.
- ❖ Never alter the angle of the twig with respect to ground while replacing the nest inside the hive.

In general, open nesting *Apis* spp. are not totally amenable for domestication. However, partial domestication of *A. florea* was attempted by a few workers with varying degree of success. (Ghatge, 1949; Pundir, 1971; Whitcombe, 1982a and 1982b; Bhamburkar and Peshkar. 1990). During the present investigation a simple low cost, card board hive was designed (Fig. 1), which served the twin purposes of colony transport and providing comfortable lodging for the bees. The large opening provided in the front wall of the hive almost created an open nest situation. In addition, the hive also provided additional protection to the colony against heat and rain. The new hive made colony inspection easy and possible at all times. Further dead bees, discarded eggs and hive debris found at the bottom floor of the hive was helpful to monitor some of the changes occurring in bee behaviour during experimentation.

5.2. Developmental biology

Production of colony members began when an egg was laid in a cell of brood comb. Inclination of egg gradually changed as the egg matured. Egg glued to the cell bottom was upright on the first day. On the second day egg was at an angle of 45^0. On the third day it was lying at the bottom of the cell. In all the colony members the embryonic development was completed in three days (Fig. 2). The larval duration lasted for four to six days (Table 3). The variations observed in the duration of larval development among the colony members may be attributed to the quality of the brood food provisioned to the various larvae. The embryonic development occurred within the egg. The larval development occurred in open brood cells as the larvae were nourished by nurse bees through progressive provisioning. The capping bees closed the brood cells with porous wax caps after the end of the larval period. Such porous brood cell cappings ensured the normal breathing process in prepupa and pupa even after the sealing of brood cells. The worm like larva metamorphosed into a pupa and later an adult bee inside the capped cells within eight to thirteen days (Fig. 2).

The developmental biology of *A. florea* was earlier studied by Sandhu and Singh (1960). The various colony members took slightly longer time (half a day to one and half days) for completing their development in Ludhiana than at Coimbatore which may be attributed to the climatic variations prevailed in the study sites. The developmental time of immature stages of honey bees are highly different among colony members. The queen developed much faster than the drones. The queen matured nearly 30 per cent faster than drones. Developmental time of queen also helped at colony level selection for rapid queen replacement as a colony without an egg layer is severely disadvantaged, by the cessation of brood rearing. The rapid development of queen at her pupal stage might be a possible cause for her faster development. As the queen larva was fed with nutritionally superior royal jelly she could develop faster than workers and drones. Drones by contrast had a leisurely development taking about 16 per cent longer from workers. Since the drone was much larger than worker, he took more time for his larval and pupal development.

5.3. Colony reproduction

5.3.1. Swarming

Swarming is a form of colony reproduction whereby the parental colony splits into one or more daughter colonies, each led by one daughter queen. Swarming helps not only in colony increase but also helps in enhancing the chances of survival through migration to new nest sites with good floral resources. Since *A. florea* is the smallest of all *Apis* species, its honey gathering potential is relatively low. In addition it cannot store a huge amount of honey as the honey storage area is small and confined to honey crest. Hence, little bees invest their biological surplus in swarms rather than storing honey that may be stolen at any time by their many enemies.

The colony reproduction in little bees mainly occurs through swarming. The development of *A. florea* colony was solely directed towards the swarming process (Fig. 6). The colony begun its growth from a cluster of broodless, combless adult bees. During swarming three to eleven queen cells were constructed along the lower edge of the comb. Queens were always produced in excess so that enough daughter queens were available to head all after swarms produced. Hence, excess queen elimination became essential for restoration of monogyny. Queen elimination occurred at two stages. The excess queens produced were destroyed either in queen cells or after their emergence. By queen elimination mechanisms all but one of the unmated queens produced during swarming were eliminated (Fig. 6). Workers played a significant role in pre emergence and post emergence destruction of queen cells in *A. florea*. Pre emergence destruction was found to be the chief queen elimination mechanism in *A. florea* (Fig. 7).

Most of the queen cells were mainly destroyed prior to the departure of final swarm by the last surviving daughter queen. The virgin queen started the queen cell destruction just by nibbling the sealed queen cells. Later the destruction was totally executed and completed by the workers. Workers preferred to damage only the sealed queen cells at the side with the tip still intact. Any damage caused to the cell led to the death of the queen pupa. After damaging the cell the workers attacked the pupa or pharate adult or ready to emerge adult dwelling inside the cell (Plate 16 & 17). Empty queen cells and damaged queen cells were left as such and were never dismantled. Cessation of nest expansion after the commencement of queen rearing and non inheritance of natal nest by daughter queen might be the possible for leaving the vacant queen cell as such.

During swarming season many queen cells were built in which the queen laid eggs at short interval which resulted in the occurrence of more than one daughter queen in the parental nest prior to their departure. The workers were also involved in post emergence destruction of virgin queens whenever the virgin queen emerged simultaneously or sequentially one after another. The excess queen elimination in *A. florea* usually occurred within the natal nest and rarely in swarm cluster. Here also workers killed the queen. Initially the workers showed hostility towards the virgin queen. Later they clustered around the queen. Finally the queen was killed due to balling (Plate 18). Dead queen found on the ground beneath the swarming colony prior to the swarm departure conclusively confirmed the occurrence of this kind of queen elimination mechanism.

The cast swarms were accompanied by more than one virgin queen rarely. During such occasions excess queen elimination also occurred in the swarm cluster. Existence of this type of queen elimination mechanism was confirmed during one occasion where one of the two queens found in a swarm cluster was missing on the second day after swarm settling. This kind of worker hostility towards the gynes was also recorded in stingless bees (Kerr, 1969; Michener, 1974; Imperatriz-Fonseca and Zucchi, 1995; Tarpy and Gilley, 2004). However, in other *Apis* spp. post emergence queen destruction always resulted due to conflict between daughter queens (Tarpy and Gilley, 2004; Gilley and Tarpy, 2005). Workers never showed hostility towards the daughter queens even though they never tolerated the presence of alien queens. The workers simply observed the queen duel and disposed of the dead queen after the queen fighting was over. Lethal queen–queen combat was a common method of queen elimination in cavity nesting *Apis* spp (Tarpy and Gilley, 2004; Gilley and Tarpy, 2005). However, in *A. florea* such combat was never observed between any of the daughter queens out of 38 daughter queens produced from five colonies during the present study.

Swarming process in *A. florea* frequently led to the dissolution of the colonies in four out of five colonies studied. The final swarm usually deserted the mother nest. In sufficient number of bees to cover the comb and aging of the comb made the mother nest unsuitable and unattractive for inheritance by the final swarm. However, in one of the colonies the final swarm stayed in the mother nest. The second swarming cycle started in the colony a month after inheritance. This colony started building many queen cells again in the centre of the comb (Plate 19) as empty queen cells previously built during the first swarming cycle were present along the lower edge of the comb. However, the second swarming cycle did not progressed till the end. A dwarf queen emerged from one of the queen cells (Plate 19). However, that queen did not survive and was also eliminated due to an unknown queen elimination mechanism.

The swarm departure from the mother nest was mediated by the piping sound produced by the virgin queen (Plate 20) and buzz runs performed by a few workers. Similar observations regarding the production of piping sound by virgin queen and its impact on swarm departure was studied by Wongsiri *et al.,* (1996) in dwarf bees. They reported cessation of worker activities due to queen piping sounds.

Functional queen which led the primary swarm and final swarm departed during during early afternoon hours (12.45 to 16.30 hours) whereas virgin queens departed during late afternoon hours (15.12 to 16. 30). A clear cut temporal separation of swarm departure was observed between functional queens and virgin queens (Fig. 4). The time gap observed between the penultimate swarm and the final swarm was fairly wider when compared with the time gap between after swarms (Table 5). As a result the last surviving daughter queen attained sexual maturity, completed mating and even started egg laying prior to her departure. Unlike in cavity nesting bees, in *A. florea* the last surviving daughter queen did not inherit the mother nest though she completed her sexual life while residing in the mother nest. Instead the last swarm also left the mother nest leading to dissolution in four out of five colomies.

The after swarms usually settled closer to the mother nest. They stayed and started a new colony closer to the parental nest. Hence, temporary nesting sites were absent in *A. florea.* The workers always remembered their old nesting site and revisited their old nest for collecting wax not only from the honey crest but also from empty queen cells (Plate 6). In this regard their behaviour was similar to stingless bees which maintained their link with mother colony for a long period for the purpose of taking away the food and resin resources from parental colony.

5.3.2. Supersedure

During the present study supersedure method of queen production was observed in two colonies for the first time. In one queenright colony there was only one supersedure cell at the time of hiving the colony from the wire fence. The cell was located at the base of the comb (Plate 21). Though the queen emerged out of the cell, the colony deserted without inheriting the mother nest for unknown reasons. The Omani method followed for hiving the nest may be a possible reason for absconding. Daughter queens produced in *A. florea* either due to swarming or emergency queen rearing did not inherit the mother nest. In the same way here also the daughter queen failed to inherit the mother nest. In another colony which was led by a queen with cut wings, many queen cells were built, as if to swarm but ended in supersedure. Here the defective queen was eliminated before the sealing of queen cells. One queen alone was allowed to emerge and all the other queen cells were destroyed before they emerge. In this situation the mother nest was inherited by the superseded queen. Occurrence of such events rarely during supersedure reported in *A. mellifera* gives support to the present finding (Wedmore, 1932).

5.3.3. Mating flights of drones and queens

Mating flight of drones in *A. florea* mainly occurred from 12.00 to 13.00 hrs in groups. Shorter duration of mating flight is an adaptive strategy for both queen and drones against predation by their natural enemies. Some drones of *A. andreniformis* ran in circling loops prior to departure. This kind of running was similar to round dance of *A. mellifera.* This dancing stimulated the group flight behaviour in drones (Wongsiri *et al.,* 1996). Such group flight reduced the probability of predation and increased the probability of mating (Wongsiri *et al.,* 1996). However, such drone dancing did not occur in *A. florea* though they flew out in groups.

Occurrence of mating flight around noon time (Fig. 5) possibly helped the bees not only to avoid bird predation as birds activity was very low or absent during that time but also helped the drones and queens to detect their avian and insect enemies easily in bright sun light. Such early mating flight also ensured temporal separation of drones of various sympatric *Apis* species and avoided mating competition from *A. cerana* and A. *dorsata* which mate during late evening and dusk respectively (Oldroyd and Wongsiri, 2006).

The timings of mating flights of *A. andreniformis* and *A. florea* were well separated temporally in Thailand, where these two species coexist. Drones of *A. andreniformis* flew out for mating after the sun passed its zenith where as drones of *A. florea* fly out for mating in the afternoon between 14.00 and 15.00 hrs (Koeniger *et al.,* 1991). During such coexistence the smaller one mated first which was also true in the above case mainly to avoid mating with larger species (Koeniger and Koeniger, 2000). Presence of drones of other species caused selection for pre mating isolation via temporal separation. On the other hand in the absence of other species the time of mating flight became extended (Otis *et al.,* 2001).

The differences in mating time and place must serve as an important behavioural barrier to interspecific mating and thus act as a mechanism to reinforce the process of speciation. Likewise copulatory and fertilization barriers prevent inter specific mating. Copulation barriers could include variation in the cocktail of chemicals in the queen pheromone and structure of male genetalia. Fertilization barriers could include failure of sperm transfer from the drone to spermatheca or death of the sperms within the spermatheca (Koeniger and Koeniger, 2000). Though *A. florea* is temporally well separated from other sympatric species, information about their spatial separation from other *Apis* spp. is still lacking. The existence of drone congregation area for dwarf bees is yet to be found out.

The male genetalia are very distinct in *A. florea*. Dorsal cornua and bulbous are quite small and pointed. They do not help in holding the queen and her parent together. Instead a thumb like process on their hind leg is useful to grasp the hind leg of the queen. The end of endophallus is not inflated into a bulb but progressively reduced into a narrow curved tube. This specially designed endophallus is useful to inject the semen directly to the spermatheca of queen. Hence, there is no wastage of semen as in *A. mellifera* or *A. cerana* where the semen is deposited in the lateral oviducts and only ten percent migrates into spermatheca. The mucous glands of drones of dwarf bees are not substantial. Hence their mating sign consisted only a small plug of orange cornual secretion (Koeniger *et al.,* 2001). In the present study also the mated queen did not have any mating sign which is in agreement with the above finding.

5.4. Colony reproduction in queenless colonies

Queen is the only female sexual reproductive of the colony capable of producing all the three colony members. The presence of queen is highly essential for the cohesive functioning of the colony. Honey bees have been described as being super organisms in which reproduction is channeled exclusively through the queen and selection on worker is restricted to maximizing their efficiency as a part of the whole (Moritz and Southwick, 1992). Queenlessness either in the colony or in the swarm markedly affects the behaviour of the workers. In the present study also this has been proved. The queenless swarms failed to form swarm clusters (Plate 10). Both the foraging and defense behaviour of the workers were affected (Table 6). The bee curtain became less compact and bees were found hanging in loose festoons (Plate 9). Colonies of dequeened dwarf bees stopped foraging. *A. andreniformis* never formed a protective curtain when the colony became queenless. The cluster broke up and bees spread out along the supporting branch in loose festoons as if they were seeking to regain their lost queen. (Wongsiri *et al.,* 1996).

If the laying queen was removed, emergency queen cells are constructed by modifying worker cells (Free and Williams, 1979; Kshirsagar *et al.,* 1983). The emergency queen cells of *A. florea* mainly occurred at the centre of the comb and were slightly shrunk beneath the comb surface and therefore difficult to locate (Sakagami and Yoshikawa, 1973). However, in our study replacement queens were reared from emergency queen cells built not only in the centre of the comb but also along the comb edge (Plate 11). But the replacement queens failed to inherit the mother nest.

The queen of a honey bee colony has reproductive monopoly because worker's ovaries are normally inactive and eggs that they do lay are eaten by other fellow workers. Workers are not sterile females but they are only non functional females in a queenless colony. They have functional ovaries but they can not mate. When colony becomes queenless many of the workers that have not yet begun foraging begin to activate their ovaries and lay male producing eggs via parthenogenesis. The haploid eggs laid by worker honey bees if properly reared they will develop into normal males fully capable of mating.

A. florea is highly polyandrous and is therefore predicted to curtail worker reproduction by mutual policing mechanism that keeps the worker reproduction at an extremely low level. Workers with activated ovaries are very rare in *A. florea* colonies (Halling *et al.,* 2001). In the presence of a queen, however any eggs that workers do lay will almost certainly be removed by other workers. Any behaviour of a worker which inhibits worker reproduction by other worker is called worker policing (Ratnieks, 1988). Policing mechanisms could be destruction of worker's eggs or their offsprings or aggression toward the reproductively active workers. Rapid removal of worker laid eggs by *A. florea* shows that this species can discriminate between queen laid and worker laid eggs. In the present study discarded worker laid eggs were found on the bottom floor of the hive (Plate 13)

Once the colony becomes queenless, workers stop policing and start egg laying, rearing the final batch of males that leave the colonies dies out. Queenless colonies are more tolerant of worker reproduction than queenright colonies. Worker egg laying is more predominant in a hopelessly queenless colony because the inhibitory influence exerted by the pheromone produced by queen and exposed brood on worker ovary development was totally absent. The number of worker laid eggs has been found to be more in hopelessly queenless colony (Plate 12). The colony finally perishes owing to the lack of replacement workers.

Queenless colonies are so reluctant to stay on the same nest because they are vulnerable to reproductive parasitism by workers from other nests. Workers from both queenless and queenright *A. florea* colonies actively seek out and parasitize queenless nests with their own eggs which is totally absent in *A. mellifera*. The workers from a queenright colony choose to parasitize queenless nests because they prefer individual reproduction rather than contributing to their own colony reproduction (Nanork *et al.,* 2005). In the present study also replacement queens produced due to emergency queen rearing did not inherit the mother nest to avoid reproductive parasitism.

Worker laid eggs appear in just three, four days, and three weeks after dequeening in *A. cerana, A. florea* and *A. mellifera* (Oldroyd *et al.*, 2001). However, worker egg laying has not been observed so far in *A. andreniformis.* A worker can remain within the natal nest, cease worker policing, attempt to lay her own eggs, and help to rear both her own eggs and those of other workers. This strategy common in *A. mellifera* (Nanork *et al.,* 2006) was also found in queenless absconding swarms of *A. florea*.

The present study has brought out some valuable information about colony reproduction in *A. florea.* However, intensification of research on the following aspects will be more helpful for thoroughly understanding the colony reproduction in *A. florea.*

- Identification of drone congregation areas
- Finding out the factors triggering swarming
- Finding out the exact causes for migratory swarming
- Evolving strategies for reducing swarming tendency
- Developing colony multiplication techniques

CHAPTER – VI

SUMMARY

Colony reproduction in *A. flora* was studied in two experimental florea apiaries established with 44 colonies during 2009 -10. The salient findings of the study are summarized below

- A low cost card board box hive suited for hiving and transporting of feral colonies of *A. florea* was designed.
- The hive so designed was also well suited for semi domestication of *A. florea* and for carrying out experimentation.
- Two experimental florea apiaries were established with 44 translocated feral colonies.
- 15 out of 44 feral colonies hived stayed for more than 100 days.
- 29 out of 44 hived colonies deserted due to queenlessness, absconding and swarming.
- Dos and Don’ts to be followed for successful hiving and transport of feral colonies were found out.
- The twig bearing the comb after removal had to be kept in the rabbets of the hive in the same angle as it was prior to removal from the tree.
- The avoidance of detachment of comb from the twig during hiving and transport minimized the desertion of the hived feral colonies.
- The time required for embryonic development was same for queen, worker and drone. The larvae hatched out within three days from the eggs.
- The queen developed faster than other colony members within 15.5 days registering shorter larval (4 days) and pupal period (8 days).
- The drone developed slower than queen and worker within 22.1 days requiring longer larval (6.1 days) and pupal period (13 days).
- The developmental time required for worker was 19 days which was more than the queen but lesser than the drones.
- The developmental time of various colony members in open cells and closed cells were almost equal.

- The swarming period commenced with drone brood rearing in December and peak period of swarming occurred during January-February.
- New queens were reared through swarming, supersedure and emergency queen rearing.
- Swarm cells were built along the lower margin of the comb. The number of cells built varied from three to eleven.
- The primary swarm was led by the old queen and after swarms except the final swarms were led by the virgin queens.
- The final swarm departure was delayed until the completion of mating by the last surviving daughter queen.
- Departure of primary swarm, cast swarms and final swarm occurred during different parts of the day.
- Swarms led by the functional queens ie., primary swarm and final swarm departed during early afternoon whereas all the cast swarms except the final swarm departed during late afternoon.
- The excess queens reared in the colony were eliminated before and after their emergence of queens from queen cells.
- Pre emergence destruction of queens developing inside the queen cells was initiated by queen but the task was subsequently completed by the workers by tearing down the queen cells laterally.
- Workers played a crucial role in the post-emergence destruction of queens in the natal nest by balling of queen.
- Queen elimination never occurred due to queen-queen conflict.
- The daughter queens were found moving on the bee curtain and produced a piping sound prior to their departure from their mother nest.
- Buzz runs performed by a few workers on bee curtain helped to recruit more workers to join the swarm.
- The final swarm departed from the nest with all remaining workers leading to the dissolution of the colony.
- The number of daughter colonies produced from a mother colony by swarming varied from two to four.
- The swarms usually settled near the mother nest without choosing any temporary nesting site.
- Peak drone flight activity occurred from 12.00 to 13.00 hrs.

- Queen mating occurred between 12.16 and 12.32 hours which coincided with in the peak period of activity of drones.
- The mated queen did not possess any mating sign.
- Drones were evicted out by workers in colonies headed by mated daughter queen.
- The queen attained sexual maturity and participated in the nuptial flight eight days after emergence.
- The workers in the swarm cluster revisited their deserted mother nest to collect wax usually from the honey crest and rarely from the base of the queen cells.
- Any injury inflicted to the queen resulted in supersedure of injured mother queen by the daughter queen which inherited the mother nest.
- Replacement queen produced by supersedure did not inherit the mother nest.
- Emergency queen cells were built either along the lower edge of the comb or from the centre of the brood nest by modifying the base of a few worker cells.
- The number of emergency queen cells built ranged from seven to thirteen when these cells were built along the lower margin of the comb whereas only two cells were constructed when the cells originated from the centre of the brood nest.
- Interrupted swarming process induced due to queen loss was revived by the requeening effort taken up by the colony by producing several emergency queen cells.
- Queenlessness in a colony resulted in increased worker aggression against intruder, decreased foraging activity and disorganized bee curtain.
- The problem of worker laying started four days after the loss of the queen.
- Worker laid eggs were either glued to the side wall of the cells or scattered all over the comb surface.
- The number of eggs laid by the worker per cell was maximum in hopelessly queenless colony (13) followed by queenless swarm (4) and queenless colony (2).
- Most of the worker laid eggs were removed and discarded on the bottom floor of the hive due to worker policing.
- Worker laid eggs did not develop into drones in queenless colonies but develop into drones in comb built by queenless swarms.

- Queenlessness did not deter the comb building activity by the bees in the swarm clusters. But they built only drone cells to rear the worker laid eggs.
- Queenless swarms stored honey in empty drone brood cells apart from storing in the honey crest region.
- Occurrence of queenlessness always resulted in the dissolution of the colony even after requeening by emergency queen rearing.

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* Originals not seen.

Table 1. Details of feral colonies of *A. florea* successfully hived

Sl. No	Place	Nesting site	Direction of nest	Girth of twig (cm)	Height from ground level (m)	Date of capturing	Residence period (up to 05.12.2010) (Days)*
1	Chinnavedampatti	*Lantana camera*	N-S	4.6	0.6	08.17.2009	268
2	Chinnavedampatti	*Coccos nucifera*	N-S	9.7	2.0	10.15.2009	204
3	R.S. puram,	*Albizzia lebbek*	E-W	2.9	4.2	12.30.2009	133
4	Udayampalayam	*Lantana camera*	NE-SW	2.2	1.8	01.28.2010	104
5	Udayampalayam	*Prosopis juliflora*	N-S	4.3	1.5	01.28.2010	104
6	Chinnametupalayam	*Azadirachta indica*	N-S	3.2	0.6	02.03.2010	98
7	Chinnametupalayam	*Calotropis* sp.	N-S	4.3	0.3	02.03.2010	98
8	Vellakinaru	*Synadenium grantii*	E-W	3.4	0.9	02.11.2010	90
9	Udayampalayam	*Azadirachta indica*	E-W	6.6	2.4	02.19.2010	82
10	Udayampalayam	*Synadenium grantii*	N-S	4.2	0.6	02.20.2010	81
11	Ganapathy	*Azadirachta indica*	NE-SW	2.2	2.4	03.20.2010	53

Table 1. Details of feral colonies of *A. florea* successfully hived (Continued)

Sl. No	**Place**	**Nesting site**	**Direction of nest**	**Girth of twig (cm)**	**Height from ground level (m)**	**Date of capturing**	**Residence period (up to 5.12.2010) (Days)***
12	Ganapathy	*Leuceana leucocephala*	N-S	2.2	3.1	03.20.2010	53
13	Vellakinaru	*Prosopis juliflora*	N-S	2.0	0.5	03.29.2010	44
14	Vellakinaru	*Azadirachta indica*	N-S	2.5	1.2	03.29.2010	44
15	Chinnavedampatti	*Synadenium grantii*	N-S	2.2	0.5	04.20.2010	22
			Mean	**3.8**	**1.5**	**-**	**98.5**

* Colonies still exist in florea apiary

Table 2. Mean residence period of deserted *A. florea* colonies

Sl. No	Cause of desertion	Number of colonies deserted	Mean residence period (Days)
1	Queenlessness	10	31.7
2	Absconding	10	33.1
3	Swarming	9	85.3

Table 3. Developmental period of queen, worker and drone of *A. florea*

Colony member	Duration of life stages (Days)			Total developmental period (Days)
	Egg	**Larva**	**Pupa**	
Queen *	3.0[a]	4.0[c]	8.6[c]	15.6[c]
Worker **	3.0[a]	4.3[b]	11.7[b]	19.0[b]
Drone **	3.0[a]	6.1[a]	13.0[a]	22.1[a]
SEd	(NS)	0.05	0.26	0.24
CD (p=0.05)		0.15	0.73	0.68

NS- Non Significant

Column means followed by same letter did not differ significantly by LSD (p= 0.05)

* Mean of 16 observations

** Mean of 30 observations

Table 4. Fate of swarm cells built in the experimental colonies of *A. florea*

Colony	**Number of queen cups built**	**Number of queen cups elaborated into queen cells**	**Transformation of queen cups to queen cells (%)**	**Number of sealed queen cells**	**Number of open empty cells**	**Number of damaged cells**	**Queen emergence (%)**
1	12	10	83.3	10	6	4	60.0
2	3	3	100.0	3	1	2	33.3
3	10	9	90.0	9	4	5	44.4
4	14	11	78.5	11	5	6	45.5
5	5	5	100.0	5	2	3	40.0
Mean	**8.8**	**7.6**	**90.4**	**5.0**	**3.6**	**4.0**	**44.6**

Table 5. Chronology of events associated with swarming in *A. florea*

Table 5. Chronology of events associated with swarming in *A. florea* (Continued)

Colony	Queen	Time of emergence (Month/Date /Time)	Fate	
			Detail	Time of occurrence (Month/Date/Time)
C_1	Mother	Unknown	Departed with primary swarm	1/16 13.30
	a	1/16 ?? ??	Captured by bee keeper	1/16 13.40
	b	1/16 ?? ??	Departed with secondary swarm	1/18 16.30
	c	1/18 16.50	Departed with tertiary swarm	1/21 16.20
	d	1/20 11.15	Killed due to balling by workers	1/20 11.30
	e	1/21 14.00	Departed with tertiary swarm	1/21 16.20
	f	1/21 16.55	Departed with final swarm	1/30 14.30
	g	-	Cell attacked by f	1/23 ?? ??
	h	-	Cell attacked by f	1/23 ?? ??
	i	-	Cell attacked by f	1/25 ?? ??
	j	-	Cell attacked by f	1/25 ?? ??
C_2	Mother	Unknown	Departed with primary swarm	1/18 14.15
	a	1/20 ?? ??	Departed with final swarm	1/30 12.45
	b	-	Cell attacked by a	1/21 ?? ??
	c	-	Cell attacked by a	1/21 ?? ??
C_3	Mother	Unknown	Departed with primary swarm	2/05 14.05
	a	2/08 17.30	Departed with secondary swarm	2/11 15.20
	b	-	Cell attacked by a	2/08 18.00
	c	2/11 16.15	Departed with tertiary swarm	2/13 15.35
	d	2/12 16.25	Dead queen found on the ground	2/13 ?? ??
	e	2/13 16.02	Departed with final swarm	2/21 12.47
	f	-	Cell attacked by e	2/14 ?? ??

Colony	Queen	Time of emergence (Month/Date/ Time)	Fate	
			Detail	Time of occurrence (Month/Date/Time)
	g	-	Cell attacked by e	2/14 ?? ??
	h	-	Cell attacked by e	2/14 ?? ??
	i	-	Cell attacked by e	2/14 ?? ??
C_4	Mother	Unknown	Departed with primary	2/01 14.09
	a	2/01 15.26	Departed with secondary swarm	2/03 16.20
	b	2/01 15.27	Unknown	2/02 ?? ??
	c	2/03 17.20	Inherited mother colony	---
	d	2/03 17.40	Unknown	2/04 ?? ??
	e	2/05 18.09	Unknown	2/06 ?? ??
	f	-	Cell attacked by c	2/06 ?? ??
	g	-	Cell attacked by c	2/06 ?? ??
	h	-	Cell attacked by c	2/06 ?? ??
	i	-	Cell attacked by c	2/07 ?? ??
	j	-	Cell attacked by c	2/07 ?? ??
	k	-	Cell attacked by c	2/07 ?? ??
C_5	Mother	Unknown	Departed with primary	2/17 13.20
	a	2/20 08.30	Departed with secondary	2/22 15.12
	b	2/22 15.19	Departed with final swarm	3/5 12.47
	c	-	Cell attacked by b	2/22 16.00
	d	-	Cell attacked by b	2/22 16.07
	e	-	Cell attacked by b	2/26 11.15

a to k : Daughter queens ?? ?? : Time information not recorded

Table 6. Colony reproduction in queenless colonies of *A. florea*

Sl. No	Behavioural parameter	Queenless colony		
		Colony 1	Colony 2	Colony 3
1	Status of the colony	Growing phase	Growing phase	Swarming phase
2	Date of loss of queen	09.10.2009	02.09.2010	03.08.2010
3	Reason for queen loss	Unknown	Accidental queen loss during capturing	Balling of queen by workers
4	Bee curtain formation	Not in order	Not in order	Not in order
5	Aggressiveness of bees	Highly aggressive	Highly aggressive	Highly aggressive
6	Bee traffic (Number / minute)	7	7	8
7	Date of appearance of emergency queen cells	09.14.2009	02.10.2010	03.09.2010
8	Number of emergency queen cells constructed	2	13	6

Table 6. Colony reproduction in queenless colonies of *A. florea* (Continued)

S.No	Behavioural parameter	Queenless colony		
		Colony 1	**Colony 2**	**Colony 3**
9	Site of construction of emergency queen cells	Centre of the comb	Along the lower comb border	Along the lower comb border
10	Date of appearance of worker laid eggs	09.14.2009	02.14.2010	03.13.2010
13	Maximum number of worker laid eggs/cell	3	2	4
11	Date of queen emergence	09.22.2009	02.20.2010	03.19.2010
12	Queen development period (days)	9	11	11
14	Number of queens emerged	1	1	2
15	Fate of other emergency queen cells	Torn down	Torn down	Torn down
16	Fate of worker laid eggs	Develop up to larval stage	Develop up to larval stage	Develop up to larval stage
17	Fate of colony after queen emergence	Absconded	Absconded	Absconded after the departure of primary swarm

Table 7. Rearing of queens under emergency impulse in *A. florae*

Major events	Colony 1	Colony 2	Colony 3
Date of queen loss	09:10:2009	02:09:2010	03:08:2010
Date of construction of emergency queen cells	09:14:2009	02:10:2010	03:09:2010
Age of the worker larva selected for queen rearing	3 days	2 days	2 days
Date of sealing of queen cells	09:15:2009	02:12:2010	03:11:2010
Date of queen emergence	09:22:2009	02:20:2010	03:19:2010
Developmental period (Larva to adult)	9	11	11

Fig. 1. Little bee card board box hive

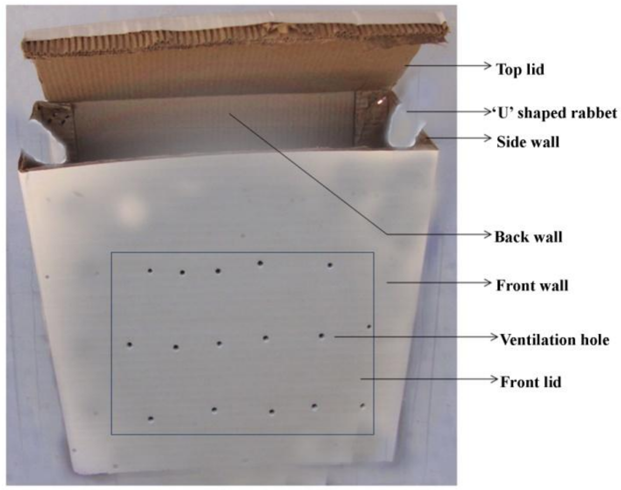


Fig. 2. Developmental period of queen, worker and drone of *A. florea*

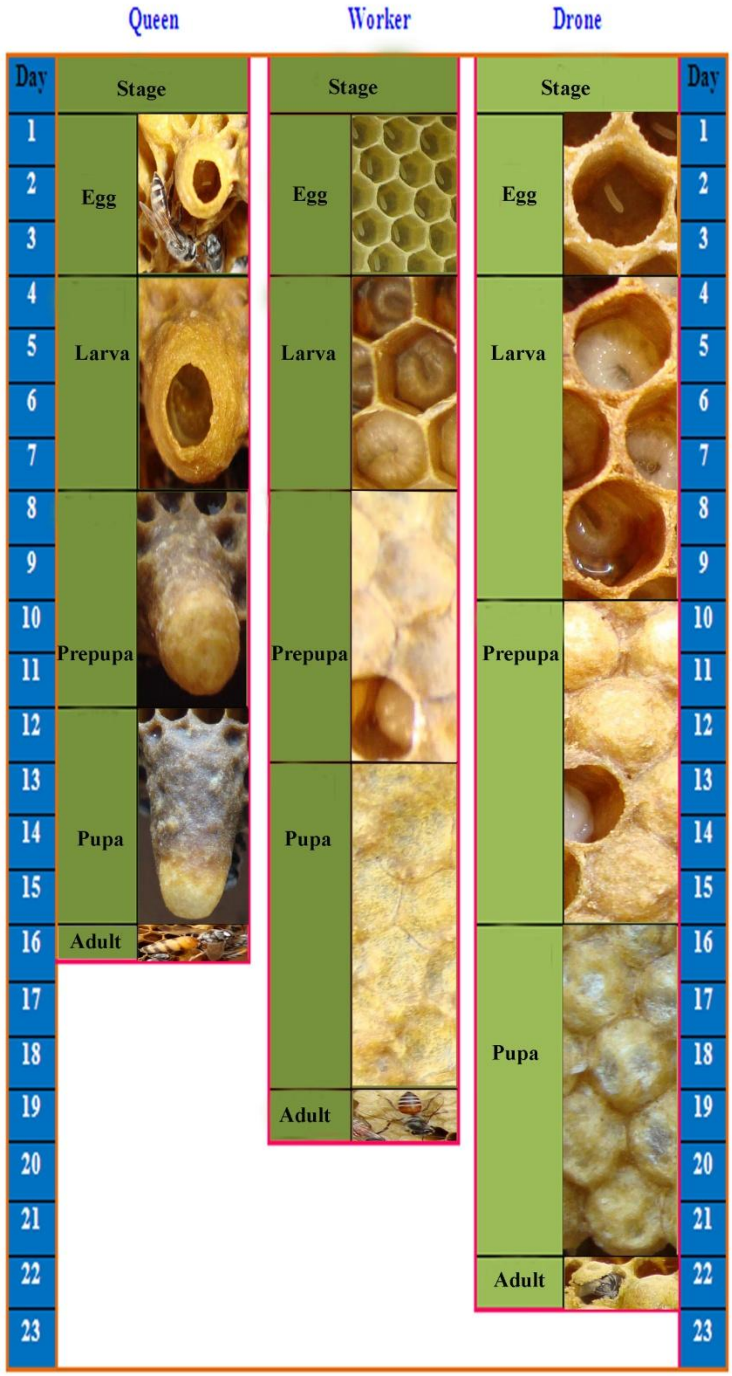


Fig. 3. Colony multiplication in experimental colonies due to swarming

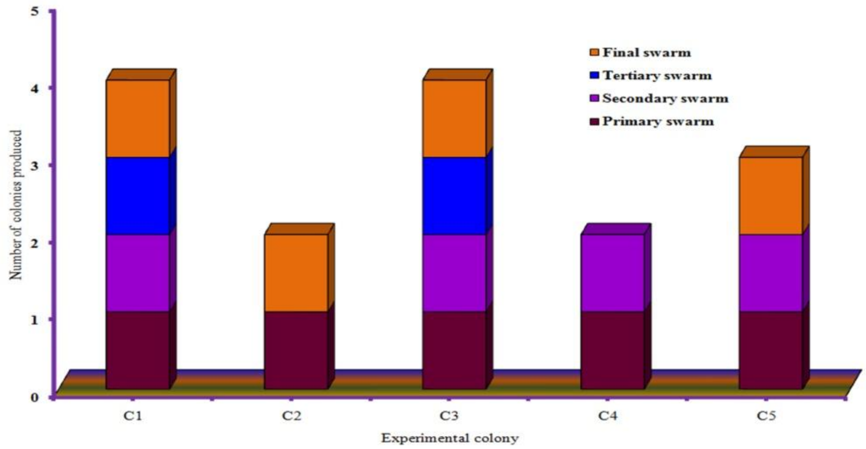


Fig. 4. Departure timings of swarms in *A. florea*

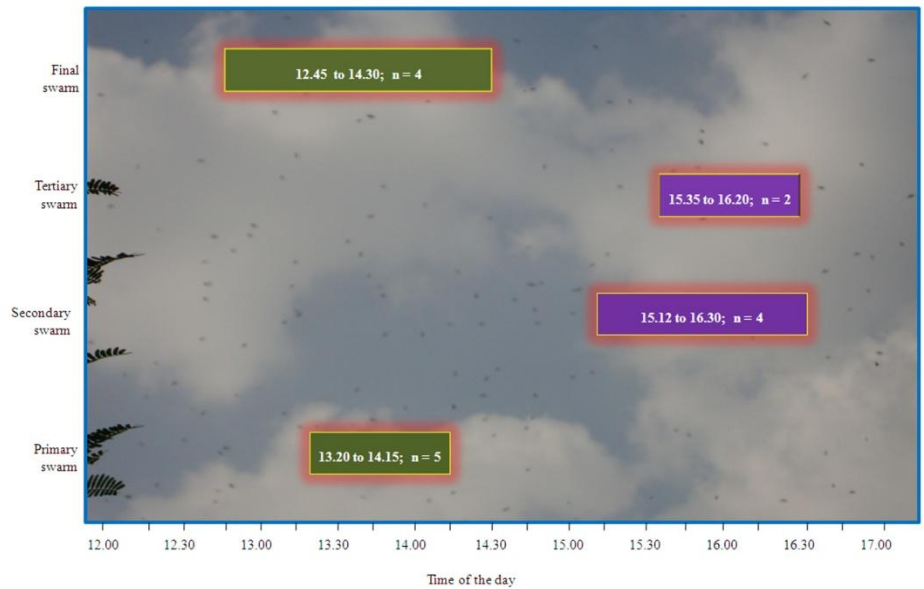


Fig. 5. Mating flight timings of drones of *A. florea*

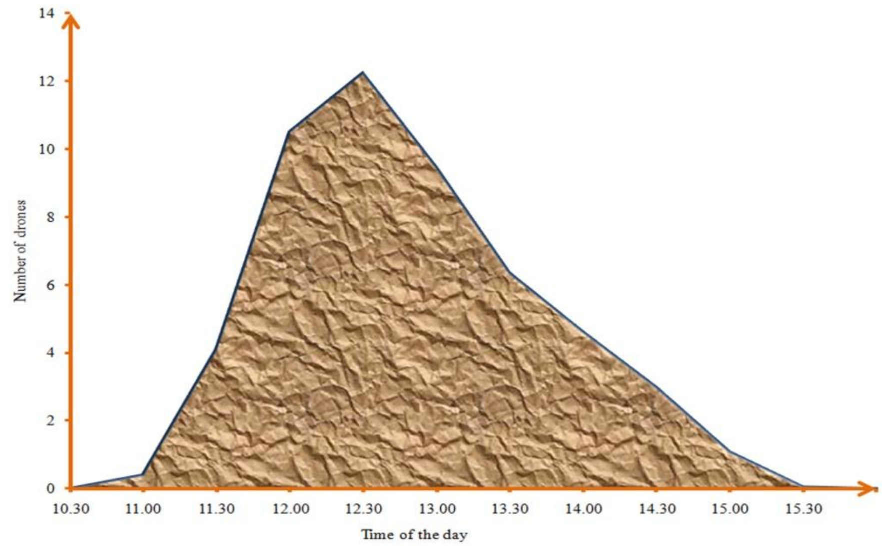


Fig. 6. Flow chart of swarming events observed in *A. florea* colonies

0

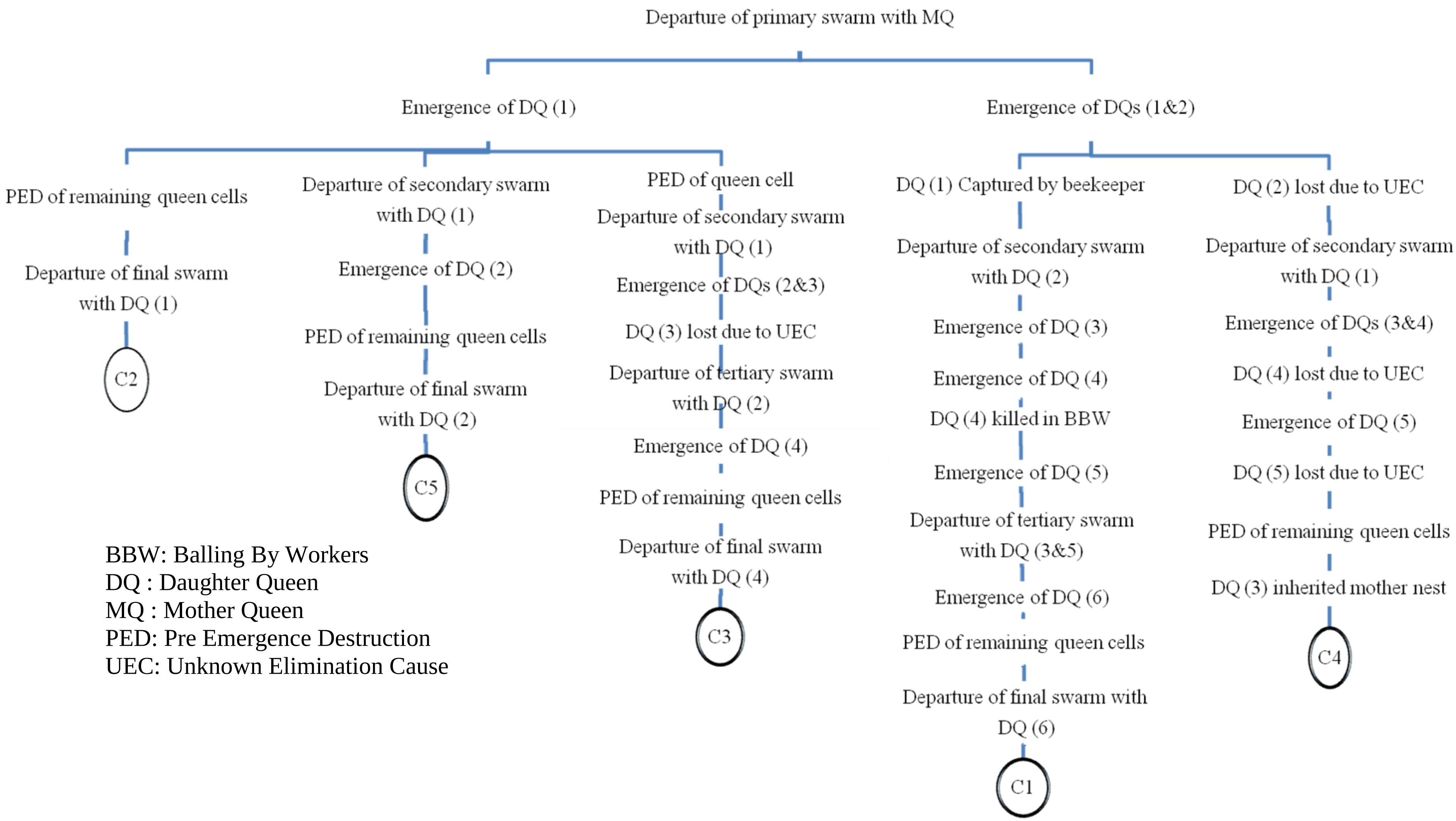


Fig. 7. The fate of *A. florea* queens reared in experimental colonies

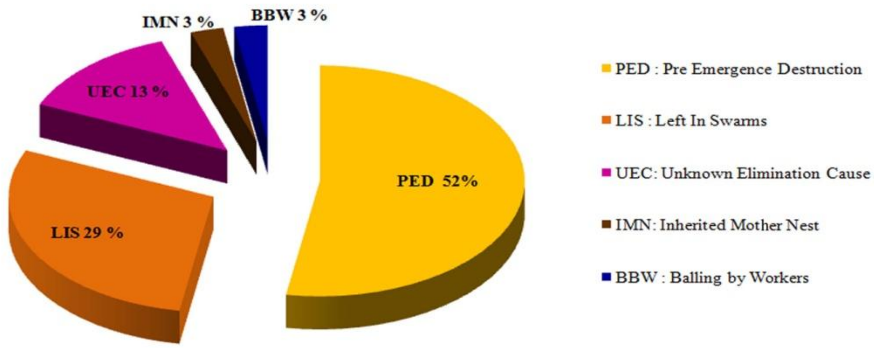


Plate 1. Experimental *A. florea* apiaries

a. Florea apiary at Ganapathy, Coimbatore

b. Florea apiary at Chinnavedampatti, Coimbatore

Plate 2. Card board box hive

a. Colony kept inside the hive

b. Hive with colony

Plate 3. Method of marking brood cells with pins

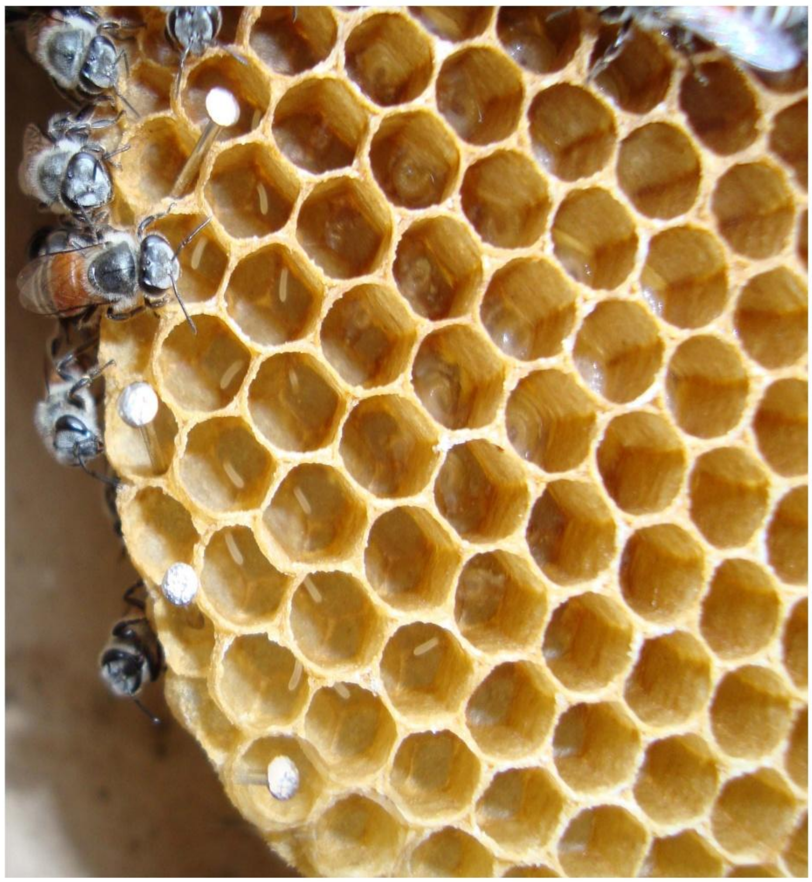


Plate 4. Swarm cells built in cluster

Plate 5. Wax removal by workers from queen cell tip

Plate 6. Worker collecting wax from empty queen cell

Plate 7. Wax moth infestation in deserted comb

Plate 8. Workers attacking a drone

Plate 9. Disorganized bee curtain of a queenless colony

Plate 10. Spread out cluster of a queenless swarm

Plate 11. Sites of construction of emergency queen cells

a. Central zone of worker brood area

b. Lower edge of the comb

Plate 12. Worker laid eggs in queenless colonies

a. Eggs glued to the side wall of the cell

b. Several eggs laid at the bottom of the cell

Plate 13. Discarded worker laid eggs on the floor of the hive

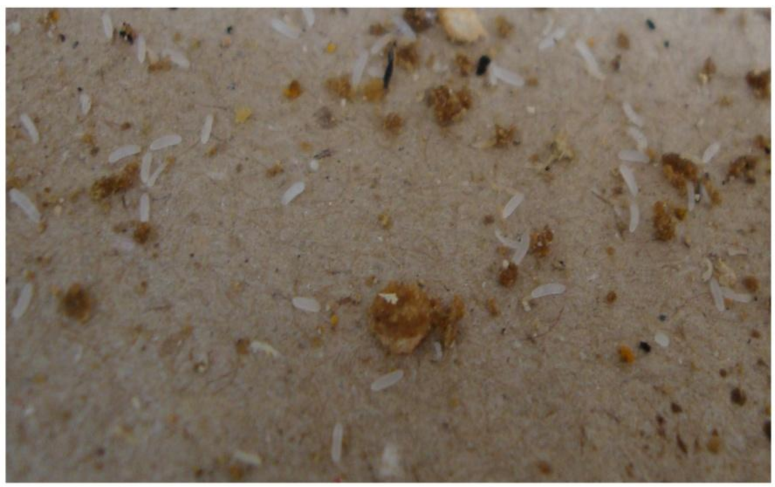


Plate 14. Worker laid eggs in drone cells

Plate 15. Honey stored in drone cells

Plate 16. Pre emergence queen cell destruction - Type 1

a. Elimination of pupa by workers

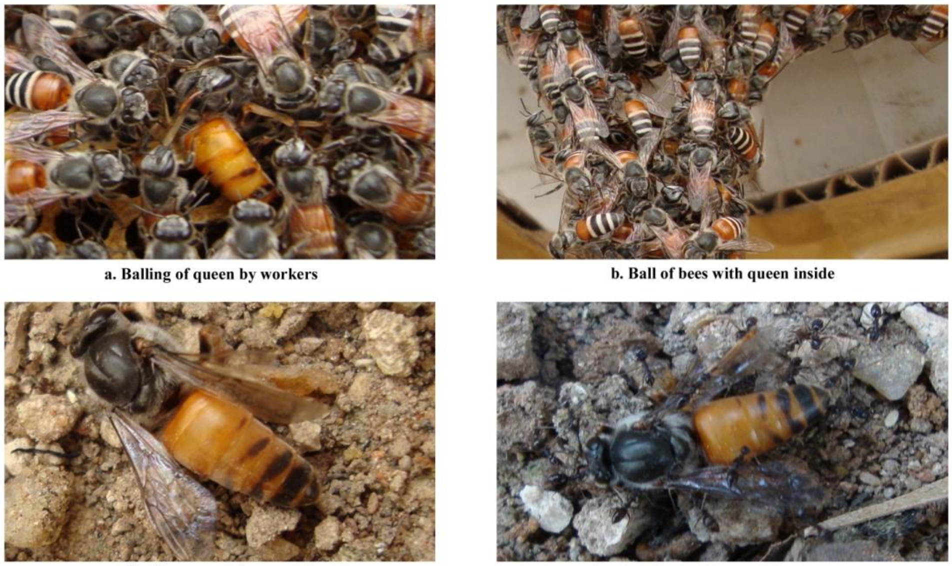
b. Elimination of pharate adult by workers

Plate 17. Pre emergence queen cell destruction - Type 2

a. Elimination of ready to emerge queen by workers

b. Eliminated queen with damaged wing

Plate 18. Queen elimination by workers



a. Balling of queen by workers

b. Ball of bees with queen inside

c. Dead queen on the ground

d. Ants attacking dead queen

Plate 19. Second swarming cycle

a. Swarm cells in the lower margin of the comb

b. Dwarf queen emerging from the queen cell

Plate 20. Position assumed by virgin queen during piping

Plate 21. Supersedure queen cell