STUDIES ON POPULATION VARIATIONS IN STINGLESS BEE *Trigona iridipennis* Smith

Thesis submitted in part fulfillment of the requirements for the award of the degree of

MASTER OF SCIENCE IN AGRICULTURAL ENTOMOLOGY *to the*

Tamil Nadu Agricultural University, Coimbatore-3

By

SRIRAM REDLA

(ID.NO.02-602-009)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

CENTRE FOR PLANT PROTECTION STUDIES

TAMILNADU AGRICULTURAL UNIVERSITY

COIMBATORE - 641003

2004

STUDIES ON POPULATION VARIATIONS IN STINGLESS BEE *Trigona iridipennis* Smith

By

SRIRAM REDLA

(ID.NO.02-602-009)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

CENTRE FOR PLANT PROTECTION STUDIES

TAMILNADU AGRICULTURAL UNIVERSITY

COIMBATORE - 641003

2004

CERTIFICATE

This is certify that the thesis entitled "**STUDIES ON POPULATION VARIATIONS IN STINGLESS BEE**" (***Trigona iridipennis* Smith**) submitted in part fulfilment of the requirements for the award of the degree of **MASTER OF SCIENCE IN AGRICULTURAL ENTOMOLOGY** to the Tamil Nadu Agricultural University, Coimbatore is a record of bonafide research work carried out by **Mr. SRIRAM REDLA** 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

Date:

(Dr. M.MUTHURAMAN)
Chairman

Approved by

Chairman

(Dr. M.MUTHURAMAN)

Members

(Dr. S.MOHAN KUMAR)

(Dr. S. SURESH)

ACKNOWLEDGEMENT

I wish to express my deep sense of gratitude to my revered chairman, ***Dr. M.Muthuraman****, Professor, Department of Agricultural Entomology for his erudite counselling, constructive criticisms and constant guidance through out the course of this investigation and the help rendered in the preparation of manuscript of this thesis.*

I am very much grateful to the members of advisory committee ***Dr.S. Mohankumar****, Associate Professor, Department of Biotechnology and* ***Dr. S.Suresh****, Professor, Department of Agricultural Entomology for their ungrudging guidance all through out the span of my research.*

I thank ***Dr. S. Palanisamy****, Professor and Head, Department of Entomology and* ***Dr. P. Balasubramanian*** *Professor and Head, Department of Biotechnology for providing all help to me during the study period.*

I wish to mention a special word of thanks to ***Dr.A. Regupathy****, Professor, Department of Agricultural Entomology, who helped me providing chemicals during my study.*

I express my sincere thanks to ***Dr. Narayanan****, Professor and Head, Horticultural Research Station, Yercaud and* ***Mr. Dhayalan Rajesh****, Planter, Hemaland Estate, Mangalam for the help extended in carrying out investigations about the hill variety of stingless bees.*

I thank ***Dr. Michael S. Engel****, Entomologist University of Kansas for identifying to stingless bees specimens.*

The junior fellowship provided by the Indian Council of Agricultural Research, New Delhi was mainly instrumental for pursuing my post-graduate study. I sincerely thank ICAR, for the same.

I am indebted to ***G.K.Thangavel*** *and* ***K. Babu*** *for all their help, comfort and affection shown during my study.*

I express my sincere thanks to ***Saravanan***, ***Subramanian*** *and* ***Geeta*** *for their active help during my research.*

I wish to place on record, my beloved one ***Miss Aruna*** *who gave me moral support indirectly during my entire course work.*

I wish to express my special thanks to my classmates and friends ***Jakka, Puppala, Bulli, Dinesh, Boopathi, Allwin, Stanly,. Radhakrishna, Bharathi, Cheliyan, Savidha, Lavanya, Priya*** and ***Kalai.***

I am in short of words in expressing gratitude to my father, mother, sisters and brother in-laws for their understanding, sacrifice, patience, support, moral boosting and encouragement during my study period.

(SRIRAM REDLA)

ABSTRACT

STUDIES ON POPULATION VARIATIONS IN STINGLESS BEE *Trigona iridipennis* Smith

By

SRIRAM REDLA

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

Chairman : **Dr. M. MUTHURAMAN, Ph.D.,**
Professor
Department of Agricultural Entomology
Tamil Nadu Agricultural University
Coimbatore-3

Research studies were carried out to find out the biodiversity of stingless bees collected from five Indian states *viz.,* Tamil Nadu, Kerala, Orissa, West Bengal and Haryana. All the specimens collected from these states were identified as *Trigona iridipennis* Smith However, the existence of limited structural variations, certain genetic variations and marked variations in the nesting biology were found out in the populations of *T. iridipennis* through this study. Distinct variations occurring in the nest entrance architecture were found out and reported for the first time. Four different nest entrance patterns *viz.,* a distinct entrance tube, an entrance tube surrounded by resin deposits, an entrance slit surrounded by large resin patch and an entrance slit with a black, circular resin ring were recognized. However, in all the nests observed the brood cells were arranged in loose clusters which is a species specific character of *T. iridipennis.* The size of the workers and dimensions of food pots were greater in bees collected from nests with entrance slits than with entrance tubes. Nest aggregation was more common in wall cavities than in tree cavities. However, nesting sites did not influence either the

pattern of nest architecture or the body structures of the worker bees. Pioneering work on DNA analysis of stingless bees brought out the existence of some genetic variations in geographically isolated populations of stingless bees. Stingless bee populations found in hilly tracts shared the same genome.

CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The stingless bees are the most abundant bees on earth. They are probably the first social bees to branch off from less social ancestors. The Meliponini is one of the older groups of bees originating in Africa and has distributed it self to all major continents, probably through the mechanism of continental drift (Wille, 1983).

Stingless bees, together with honey bees, attained the highest level of social evolution among the bees in general. In stingless bees and honey bees, queens and workers are more diverged in their morphology and more specialized in their tasks (Velthuis, 1997). They live in permanent colonies and multiply through a process called swarming.

Stingless bees are a group of small to medium sized bees. They have reduced wing venation and sting, which is compensated by their stout mandibles supported by strong muscles. They cannot sting because in these bees the sting is vestigial without an effective tip and they have no venom apparatus. Hence, the defense behaviour of stingless bees is much less hurtful than that of honey bees. They chase away the intruders by biting. These bees are sometimes called dammer bees as they collect a kind of resin for constructing their nest along with wax produced from their body. They live in permanent colonies. They build their nests either in a wall cavity or a tree cavity. The nest is usually made of five parts; brood comb, involucrum, store pots, batumen and an entrance. The comb consists of brood cells, in each of which a single young bee is reared, and surrounded by a sheath of cerumen, or involucrum. Cerumen is made of a mixture of wax secreted from the glands on the abdomen of workers and propolis, which is derived from resins collected from plants. Honey and pollen are stored in pots quite different from the brood cells. Batumen plates usually made of cerumen and mud seal the extra space in the wall cavity or tree cavity. The entrance of the nest is a simple hole.

It often extends from the nest as a tube, and also continues inside. There are pillars and connectives inside the nest to support all the other structures within the batumen plates. Cluster type nests can take advantage of small and irregular spaces (Michener, 1974).

Melipona and *Trigona* are the most important genera of stingless bees. They occur in the tropics and sub-tropics of South America, South Africa and South East Asia. Over 500 species of stingless bees are known and majority are in South America. *Trigona* is a group of stingless bees characterized by small body size, reduced sting and wing venation and highly developed social structure comparable to that of honey bees. *Trigona iridipennis* Smith was originally described in 1854 from Sri Lanka which is found in Asiatic main land. *Trigona* refers to the triangular shape of the bee's abdomen and *iridipennis* refers to their iridescent wings.

The most common species of stingless bees found in India was first described as *Melipona iridipennis* by Smith. However, it is widely accepted now that species belongs to the genus *Trigona* and not *Melipona*. *Trigona iridipennis* was redefined as the species belonging to India and Sri Lanka (Sakagami, 1978). The species found in Bangalore area (Biesmeijer, 1993) and in Kerala (Raakhee mohan and Devanesan, 1999) were identified by Roubik as *T. iridipennis*. The species occurring in Tami Nadu was identified as *T. iridipennis* (Swaminathan, 2000). A thorough study on the species diversity of *Trigona* in different parts of India still lacking. Hence, the present study was taken up with the following objectives.

- To find out the population variations of stingless bee *T. iridipennis* collected from different geographical locations through morphometric studies.
- To investigate the nesting biology of stingless bee *T. iridipennis* found in different parts of Tamil Nadu by nest dissection studies.
- To trace out the existence of genetic variations of *T. iridipennis* if any through DNA analysis.

CHAPTER II

REVIEW OF LITERATURE

2.1 Species of stingless bees

Stingless bees are eusocial bees. They are known for their rich biodiversity. They constitute a diverse group with about 500 species (Roubik, 1992) which occur in all continents. They are taxonomically different from honey bees. Honey bees belong to the sub family Apinae, while stingless bees are grouped under the subfamily Meliponinae. Meliponinae is easily distinguished from other subfamilies of Apidae by the reduced wing venation, presence of penicillum and vestigial sting (Wille, 1979,1983; Michener, 1990, 2000). A major character that separates meliponini from other corbiculate tribes is the absence of an auricle on the hind basitarsus. These bees are organized taxonomically in to two tribes *viz.*, Trigonini, the largest group with various genera and Meliponini a tribe consisting exclusively of the genus *Melipona*. Meliponinae is restricted to neotropics (Michener, 1974). They are especially diverse and abundant in tropical America. The genus *Melipona* consists of about 40 medium to large size bee species (Camargo *et al.*, 1988). The species include in this tribe shows considerable variation in size, nesting site and nest architecture (Michener, 1974; Sakagami, 1982).

All Asian and African stingless bees belong to the tribe Trigonini. Sixty species of stingless bees belonging to 14 genera are found in South East Asia. The various genera in this tribe include *Tetragonula*, *Trigona*, *Plebeia* and *Nanotrigona*. Taxonomic features of *Tetragonula* were given by Moure (1961). Sakagami (1978) divided *Tetragonula* in to four species groups *viz.*, *tetragonula* group, *iridipennis* group, *laeviceps* group and *carbonaria* group. Each species group included a number of very similar species that could be recognized based on morphometric studies (Sakagami, 1978; Sakagami and Inoue, 1985). Further more, many *Tetragonula* species occur in sympatry, often

aggregating their nests at the same place (Starr and Sakagami, 1987). Two genera of stingless bees occur in Australia. They are endemic *Austroplebeia* and the most cosmopolitan *Trigona*. The five Australian *Trigona* species belong to the subgenus *Tetragonula* which is considered to be part of the subgenus *Heterotrigona* (Michener, 1990). *Tetragonula* is the largest subgenus of Indo-Pacific stingless bees and includes around 20 species, some of which are very common and widespread. Later *Tetragonula* has been divided into five species groups *viz., geissleri, clypearis, laeviceps. Fuscobalteata* and *carbonaria* (Dollin *et al.,* 1997).

Trigona is the largest and most widely distributed genus with neotropical *Trigona* and most of the Asian Meliponini (Velthuis, 1997). They are abundant in the forests of tropical and subtropical regions. The most common species of stingless bees found in India was known by the name *Melipiona iridipennis* Smith Later the taxonomic confusion avoided by placing the species *iridipennis* under the genus *Trigona* as *Melipoina* is restricted to only neotropics (Michener, 1974).

2.2 Nest architecture

More general information on nest architecture in stingless bees is given by Kerr *et al.,* (1967), Nogueira-Neto (1970), Michener (1974), Sakagami (1982) and Roubik (1983,1989). The nest of stingless bees usually consists of an external entrance tube, internal tunnel, resin dumps, waste dumps, food pots for pollen and honey, brood cells and nest envelopes like involucrum and batumen. Brood cells and food pots are made of cerumen, which is mixture of wax and resin. The cells of the combs are in contact with one another, some times however they are connected by small pillars or connectives of soft cerumen. Pillars are vertical and connectives are more or less horizontal. Brood cells, honey pots and pollen pots are arranged in separate clusters (Pooley and Michener, 1969). Currently, several of these *Trigon* species are most readily distinguished from each other by characteristics of their nest structure (Dollin *et al.,* 1997).

2.2.1 Nesting site

Stingless bees are cavity nesting bees. Tree cavities are the most common and predominant nesting sites of stingless bees. Hollows inside living or non living trees or under root systems are used for nesting. *T. carbonaria* species were typically found in coastal areas of eastern Australia, where nests are usually established in hollow trees and logs (Michener, 1961; Heard, 1988; Dollin *et al.,* 1997). *T. gribodoi* Magetti nests in cavities of tree trunks and branches (Pooley and Michener, 1969). Sumatran stingless bee, *T. laeviceps* Smith often nests in hollow trees or eaves or pillars of wooden houses (Sakagami *et al.,* 1983a). Some times their nests are exposed. Such exposed nests are entirely or partially surrounded by hardened resin called batumen. In the fully exposed nests the layers of batumen are numerous and mostly strong and rarely brittle. Batumen is laminate when the nest is exposed. Spaces between batumen layers are filled with bee's faces or earth or vegetable fibres. The filling may be total as in *T. corvine* or partial in *T. spinipes* Fabr. (Wille, 1983).

Species that build their nests in underground cavities and among the roots at the bases of trees are relatively few among stingless bees. They may nest in soft or hard soil (Pooley and Michener, 1969). They construct either long or short drainage tubes to drain away excess water from their nest (Wille, 1966).

T. sapiens builds their nest inside the stone walls, under concrete foot path or in natural rock crevices. Nests of *T. clypearis* were commonly found in building cavities including hollow walls or doors. Nests were rarely found inside iron pipes (Dollin *et al.,* 1997).

T. oyani Darchen builds its nest within the ant nest of *Crematogaster spp*. using cavities produced by depredation of the diurnal pangolin (Darchen, 1971). Some species of stingless bees use abandoned *Atta* nests as a response to available cavities in the soil. *T. moorei* Schwarz is obligatiorily myrmecophilous and found with in active arboreal ant

nests of *Crematogaster sp*.(Sakagami *et al.,* 1989). *T. denoiti* Vavhal. builds combs generally inside the nests of termites (Smith, 1954). A colony of *T. sawadogoi* Darchen .was found in a cavity about 13 cm in diameter inside a live termite nest .The bees nest was separated by a hard resinous shell. The horizontal combs were constructed from bottom upwards (Darchen, 1970; Wille and Michener, 1973). Nesting association with termite colonies (*Nasutitermes*) was noted for *T. pallens* (Roubik, 1979).

2.2.2 Nest entrance

The nests of stingless bees are often conspicuous by the entrance tube through which bees enter and leave. Each nest has just one tube made of cerumen. Multiple entrance tunnels are occasionally observed in *T. mellipes* of which part of them are used while the remaining are disused (Dollin *et al.,* 1997). Its length varies widely. The entrance usually consists of a simple crack or hole about one cm wide in a tree trunk in *T. hockingsi* Cockrell. The entrance tube is slender and sticky in *T. moorei* (Sakagami *et al.,* 1989). The external entrance tubes of *T. gribodoi* are 6-25 mm long and project at an angle to the bark (Pooley and Michener, 1969). The entrance tube apertures ranged from slit like to elliptical in *T. sapiens* and approximately spherical in *T. fuscobalteata.* They are distinctly longer and narrower in *T. sapiens* than *T. fuscobalteata* (Starr and Sakagami, 1987).

The bees often build a passage way or internal tunnel leading from entrance to the inner parts of the nest. A resin dump is found in this area to supply the guard bees with sticky weapon to fight off intruders. They also use the resin as a building material for nest construction.

T. carbonaria and *T. hockingsi* nests normally lack external entrance tunnels. However, these species some times reduce a large hole to a smaller entrance with a flat sheet of cerumen. In *T. carbonaria* nests the entrances and surrounding areas were coated

with a smooth, thick layer of cerumen, which may be black, red or yellow. *T. hockingsi* nests had large areas daubed with globular mixtures of resin and other materials. In *T. dorsalis* also external entrance tube is absent (Wille and Michener, 1973).

2.2.3 Food pots

According to the spatial limitation of the nest cavity, the storage pots are found either above, below or at both sides of the brood area. Food pots are found amongst the scaffolding network of cerumen sheets and pillars. More food pots are built one over the other or side by side. When full they are sealed, the whole collection looks like a densely packed bunch of grapes. Pollen pots are built closer to the entrance. Blackened and useless pollen was found in the nests of several species, typically those having large pollen stores (Roubik, 1983). *T. hypogea*, an obligate necrophage did not collect pollen (Roubik, 1983). Honey pots are commonly found in the outer parts of the nest but often a cluster will contain both honey and pollen pots. (Dollin, 1996).

2.2.4 Brood cells

Brood cells tends to be more crowded. The advancing front is the surface where new cells are added to brood. Multiple advancing fronts are also common. Brood cells are vertically elongated and oriented. They are mostly arranged in horizontal layers forming combs. But in some groups, cells are arranged in clusters. Sakagami *et al.*,(1983b) studied the nesting behaviour of nine species of Southern Asian stingless bees and found that in *T. apicalis* Smith and *T. thoracica* Smith brood cells were arranged in combs.

The brood chamber of *T. gribodoi* consists of ten horizontal combs (Pooley and Michener, 1969). Brood combs of *T. denoiti* are spirally arranged in single or double spirals. Spirals rotate either clockwise or anti-clockwise (Fletcher and Crewe, 1981). *T. staudingeri* Gribodoi builds vertical double sided combs like those of *Apis mellifera*

which is unique among Meliponinae (Smith, 1954). Brood cells are arranged in clusters in *T. fuscobaltiata* Cameron (Starr and Sakagami, 1987). *T. nigra pauperra* Provencher constructs its nest in a cluster formation (Sommeijer *et al.*, 1984).

In *T. clypearis* the brood cells are arranged in clusters. The brood cells in the nest are vertically elongated and roughly arranged in diagonal rows. However, they lack any comb structure. In *T. sapiens* nests had cluster like broods, but groups of larval cells are reinforced with cerumen to form irregular horizontal or diagonal layers. In *T. carbonaria* the brood cells constitute a spirally built single layer of concave to flat hexagonal combs (Dollin *et al.*, 1997).

Brood cells house eggs and larvae. The larval brood cells are built of brown cerumen. Prior to pupation the larvae spin a silken cocoon. In *T. clavipes* the larval and pupal cells are nearly identical (Roubik, 1979). Much of the brood cell cerumen is then stripped away leaving the cocoons largely exposed. Queens are reared in special cells. These cells are large and found on the edge of the comb. In *Trigona* spp. the worker cells and drone cells are similar (De Bruijin, 1994).

2.2.5 Nest envelopes

Batumen

The cavity is often limited by special plates called batumen usually made of hard cerumen. The walls of the nest cavity are frequently lined with a thin layer of cerumen called lining batumen (Michener, 1964). The batumen gives protection against possible enemies and also serves as a protection against water (Velthuis, 1997). Extremely thick batumen envelops of resin enclosed subterranean nests of *T. fulviventris* and nests of *T. hypogea* (Roubik, 1983)

Involucrum

A single or multiple layers of soft cerumen called involucrum may surround and protect the brood cells and cocoons

2.2.6 Waste dump

Another intranidal characteristic is the occurrence of distinct waste dump where workers defecate and deposit the remains of cocoons and dead bees (De Bruijn, 1993).

2.3 Bee DNA Studies

Molecular markers *viz.,* random amplified polymorphic DNAs (RAPDs), restriction fragment length polymorphism (RFLPs) and microsatellite DNAs are commonly used for studying the population biology, pattern of inheritance, genome mapping, behavioural genetics, phylogeny, evolution, and biodiversity of both honey bees and stingless bees (Fondrk *et al.,* 1993; Hunt and Page, 1992, 1995; Waldschmidt *et al.,* 1997; Francisco *et al.,* 2001; Costa *et al.,* 2003).

RAPD is ideally suited for studying honey bee genetics since it is a simple and straightforward PCR based technique which does not require previous knowledge about the sequence being amplified. RAPD markers have been successfully used to determine the parentage (Fondrk *et al.,* 1993) and construct linkage maps in *Apis mellifera* (Hunt and Page, 1995). These markers are extremely useful for genetic and taxonomical population studies (Suazo *et al.,* 1998). These authors detected specific RAPD markers which were able to distinguish the African and European races of *Apis mellifera*. These RAPD markers could also be used to study the genetic structure of hybrid zones where populations differing by a few or several characteristics intercross (Futuyma, 1993). These zones can provide important evolutionary information concerning the degree of genetic introgression, the way barriers for genetic exchange work and also the genetic nature and ecological differences between species (Harrison, 1990). Waldschmidt *et al.,* (2000)

had identified a RAPD marker useful to distinguish the subspecies of stingless bees *viz.*, *Melipona quadrifasciata quadrifasciata* and *Melipona quadrifasciata anthidioides* and studied the genetic structure of the hybridization zone formed by the intercrossing of two subspecies.

Task specialization in little bee (*Apis florea*) has been confirmed by Oldroyd *et al.,* (1994) based on the variations found in RFLP banding. They have proved the existence of genetic components in the task division of this species.

Microsatellite markers with typically high levels of polymorphism may be useful in determining relatedness among colonies, estimating levels of inbreeding and assessing population structure. They could also be utilized in investigations of unstudied aspect of sociobiology of *Trigona* species. Microsatellite loci have been reported from *Melopona bicolor* (Peters *et al.*,1998) and *Scaptotrigona postica* (Paxton *et al.,* 1999). Microsatellite loci were isolated from *Trigona carbonaria* following the screening of a partial genomic library with several simple repeat oligonucleotide probes (Green *et al.,* 2001).

Mitochondrial DNA (mt DNA) is one of the most widely used molecules in systematic, species characterization, population structure and phylogenetic studies in *Apis mellifera*. Polymorphism of *Apis* mitochondrial DNA has provided markers that have been extremely important for the study of population, hybridization, species and subspecies. Intraspecific genetic variations existing among five species of *Plebia* were found out by the characterization of mitochondrial DNA by RFLP analysis and restriction maps (Francisco *et al.,* 2001). Molecular phylogeny of stingless bees was studied using mitochindrial 16S rDNA sequences (Costa *et al.,* 2003).

CHAPTER III

MATERIALS AND METHODS

The present study was mainly taken up to find out the variations existing in the populations of *Trigona iridipennis* Smith and the materials used and methods employed are given in this chapter.

3.1 Nest aggregation

Studies on nest aggregation was carried out in a dilapidated tiled house (Plate 1) located in a village near Sirumugai. The total number of colonies found inside the walls were counted by counting the number of entrance tubes found on the walls. Variations observed in the nest entrance architecture were recorded. Metric data was gathered pertaining to the length and diameter of the entrance tubes. Number of colonies having single entrance and multiple entrance (double or triple) were counted and recorded. Details were also collected regarding the orientation of the entrance tubes.

3.2 Nest architecture

3.2.1 Nest entrance architecture

Details pertaining to variations in nest entrance architecture were collected by observing the presence or absence of entrance tubes. In addition, the presence of resin, its nature and pattern of its distribution around the nest entrance was studied by visual examination.

3.2.2 Variations in nest components

Nests showing deviations in nest entrance architecture were dissected to study the internal arrangement of different nest components especially the brood cells and food pots. Metric data was collected pertaining to the food pots and brood cells.

3.3 Morphometric studies

Adult stingless bees of *T. iridipennis* were collected both from natural colonies and colonies kept in artificial enclosures having a distinct entrance tube from nine different geographical locations *viz.,* TNAU campus, Malaimandiri Palayam, Yercaud, Iruttupallam, Theerthamalai, Pala, Bhubaneswar, Bir Bhum and Hissar situated in five different states i.e. five from Tamil Nadu, and one each from Kerala, Orissa, West Bengal, and Haryana respectively. Out coming worker bees were collected by tapping the region around the nest entrance in test tubes. The collected specimens were preserved in 70% ethyl alcohol. Similarly worker bees were collected from nests having different nest entrance architecture.

Morphometric observations pertaining to 27 structural characters were taken in five workers from each population by using image analyzer to differentiate them structurally. Permanent slides were prepared for various body regions/parts *viz.,* head, antenna, wings and legs using standard procedures.

The image analyzer used in the studies consisted of a stereoscopic microscope attached with a CCD camera and a computer fitted with Biovis imge software (Plate 2). The length and width of the various body regions and selected body parts (Table 9) were easily measured using the image analyzer and the images were stored in the computer for future reference.

3.3.1 Statistical analysis

Data on structural variations existing among different populations were analysed using completely randomized design. For making comparisons either paired ‘t’ test or independent ‘t’ test was employed.

3.4 Random Amplified Polymorphic DNA analysis of *T.iridipennis* population

It involved two major steps *viz.,* isolation of genomic DNA and PCR analyses of DNA using insect specific nuclear primers.

3.4.1 Isolation of genomic DNA

Stingless bee populations were collected from different parts of Tamil Nadu *viz.*, Coimbatore, Sittlingi, Theerthamalai, Iruttupallam and also from kerala. Ten workers collected from each place were ground with 2 ml of CTAB (Cetyl Trimethyl Ammonium Bromide) buffer containing 4% CTAB in 100mM of Tris-HCl of pH 8.0, 1.4mM of Sodium chloride, 20mM of EDTA, 0.5M of Glucose, 2-Mercaptoethanol added just prior to use at 0.1%. The suspension was incubated at 65°C for 2 h and then equal volume of Chloroform: Isoamyl alcohol (24:1) was added. The suspension was centrifuged at 15000 rpm for 15min at 4°C. The supernatant was transferred to a fresh tube and DNA was pelleted by adding 0.7 volume of ice-cold isopropanol and centrifuged at 10000 rpm for 15 minutes at 4^0 C. The DNA pellet was dissolved in 50 µl TE (Tris EDTA, 100mM). To the well dissolved DNA, 5 µl of RNAse was added and incubated for one hour at 37^0 C.

3.4.2 PCR amplification

The genomic DNA from five different populations collected from five different locations were subjected to polymerase chain reaction (PCR) with five insect specific nuclear primers (Table 1) obtained from NAPS Unit, The University of British Columbia, Vancouver, Canada. Amplification of genomic DNA was carried out in 20 µl reaction mixture containing 40 to 50 ng of DNA as template. The constituents of the reaction were sterile distilled water (12.70 µl), 2.5mM of dNTPs (0.8 µl), 10X assay buffer (2.0 µl), 20µM of RAPD primer (2.0 µl), 25ng of genomic DNA (2.0 µl), 1unit of *Taq* polymerase (0.25µl) and 25mM of Magnesium chloride (0.25 µl). DNA amplification was performed in a PCR thermal cycler (MJ, Research. Inc., USA) programmed for 1 cycle of initial

denaturation at 94º C for 5 min followed by 34 cycles at 94ºC for 1 min denaturation, 55ºC to 59ºC according to the primer used for 1 min annealing temperature, 72º C for 2 min extension, followed by final extension at 72ºC for 5 min.

3.4.3 Gel documentation

Amplification products were analyzed by electrophoresis on 1.5% agarose gel (w/v) in 1x TBE and photographed using Multi Images™ light cabinet with Polaroid camera attachment and documented using AlphaEaseFC™ software Version 3.2.1 of Alpha InfoTech Corporation Inc. One kilo-base DNA ladder (Fermentas, USA) was used as the molecular size marker.

3.4.4 Scoring of bands and construction of dendrogram

The bands were scored with the presence of bands (1) and absence of bands (0). The scored bands were fed in an excel sheet and the similarity index was worked out. Cluster analysis and dendrogram were constructed using NTSYS pc-2.0 software programme and in it, SHAN clustering was chosen for the analysis (Stevens and Wall, 1995; Lynch and Milligan, 1994).

CHAPTER IV

RESULTS

The results pertaining to the variations observed in the different populations of *T. iridipennis* with reference to nest aggregation, nest architecture, morphometric studies and DNA analysis are presented below.

4.1 Nest aggregation

A total of 111 colonies were found in the wall cavities of the building. In all the colonies a distinct entrance tube was found. Out of 111 colonies nine colonies had multiple entrance tubes. Seven colonies had double entrance and two colonies had triple entrance (Table 2). The length (0.9 to 10.5) and diameter (0.4 to 1.8 cm) of the entrance varied widely (Table 3). The entrance was mainly designed by using resin and mud (Plate 3). In general the exterior of the entrance tube was roughly circular (Plate 3). In some nests the entrance tube was dorsoventrally compressed (Plate 4). The number of guard bees guarding the nest entrance varied with the diameter of the nest entrance. A maximum of 16 guard bees was found in one nest (Plate 4). In one nest the entrance tube was unusually long (Plate 5). The orientation of the entrance tube varied widely. In many cases it was parallel to the ground (Plate 6). In very few cases the entrance tube was inclined upwards at an angle (Plate 7) or pointing downwards (Plate 8). Usually the entrance tube contained single tunnel for the bees to enter. Rarely entrance tubes with two (Plate 9) or three (Plate 10) tunnels were also observed. The gradual development of a double tunnel from a single tunnel is depicted in Plate 11. In general all the tunnels were put to use but sometimes one tunnel was actively used while the other one was sealed (Plate 12). Out of 111 colonies in two colonies the entrance tube was very short, wide and perfectly circular in outline with a prominent resinous rim (Plate 13).

4.2 Nest architecture

4.2.1 Nest entrance architecture

This study has documented for the first time the variations in the nest entrance architecture. In majority of the *T. iridipennis* colonies observed, a prominent entrance tube of varying length was found (Plate 14). In one location at Yercaud hills (Hemaland estates, Mangalam) in five colonies found inside stone wall cavities a distinct entrance tube was totally lacking. The narrow slit found in between the stones was mainly used for the entry and exit of the bees. The entrance slit was surrounded by a large resinous, greasy patch of size 15510 cm (Plate 15). A few black ants (*Componotus compressus* Fabricius) were found trapped and killed in the peripheri of resinous region. In three colonies the resinous patch was dry and straw yellow in colour (Plate 16).

In another location at Anthiyur in one colony found inside a wall cavity in addition to a distinct entrance tube dried resin deposits were seen scattered around the entrance tube (Plate 17).

Another deviation in the nest entrance architecture was observed both in colonies found inside wall cavities at Sirumugai and tree cavities at Sittlingi. In these colonies the entrance tube was almost absent. The nest entrance was perfectly circular. The entrance was surrounded by black, circular resin ring (dia 1.8cm) (Plate 13).

4.2.2 Variations in nest components

Dimensions of food pots and brood cells did not vary significantly between nests with a distinct entrance tube and nest with an entrance tube surrounded by resin deposits (Table 4). Honey pots and pollen pots were significantly wider and larval and pupal brood cells were significantly longer in nests with nest entrance surrounded by large resin patch (Table 5) and nest entrance with circular resin ring (Table 6) than in normal nests. Dimensions of brood cells did not vary significantly between nests with a circular resin

ring at the nest entrance and nest with nest entrance surrounded by large resin patch (Table 7). Nest location either inside a tree cavity or a wall cavity did not influence the dimensions of either brood cells or food pots in nests with nest entrance surrounded by circular resin ring (Table 8).

4.2.3 Brood cell arrangement

Nest dissection studies taken up revealed that there was no variations in the pattern of arrangement of brood cells and food pots in colonies with four different patterns of nest entrance architecture. In all these colonies the brood cells were arranged in loose clusters (Plate 18 to 20).

4.3 Structural variations

Morphometric variations based on 27 characters observed among the workers collected from nine different geographic locations from nests with characteristic entrance tubes are tabulated in Table 9. In general the size variations observed among different populations were limited i.e. the body length, antennal length and abdominal length were similar in eight out of nine samples. Head size (length and width) and the size of the pollen comb remained constant in all the samples.

Comparative variations recorded between workers collected from nest with distinct entrance tube and nest with entrance tube surrounded by resin deposits (Table 10) clearly indicated the absence of variations pertaining to structural characters except the total body length, eye length and inter ocellar distance.

Structural characters were compared between workers collected from normal nests with distinct entrance tube with workers collected from entrance slit surrounded by large resin patch (Table 11) and entrance slit surrounded by circular resin ring (Table 12). The workers bees collected from the latter two nests were larger in size than those

collected from the former nests. These bees had a longer body and larger body parts. They had larger head, larger compound eyes, longer antennae, wider thorax, larger pollen baskets, pollen combs and wings.

Similar structural variations were observed between workers collected from entrance slit surrounded by large greasy resin patch and entrance slit surrounded by dried resin patch (Table 13). Body and mandible length of workers collected from nests with entrance slits surrounded by large greasy resin patch were 5.36mm and 0.80mm respectively, whereas in workers collected from nests with nest entrance with circular resin ring were 5.87mm and 0.69mm respectively (Table 14). Nest entrance architecture remained the same in nests found either inside a wall cavity or a tree cavity. Similarly the structural variations observed between the workers collected from nests with nest entrance surrounded by circular resin ring found either inside a wall cavity or a tree cavity were minimum (Table 15).

4.4 DNA variations

4.4.1 Random Amplified Polymorphic DNA analysis of *T. iridipennis* populations

Five lepidopteran specific random primers were used to study the inter population variations in *T. iridipennis* populations. The bee samples were collected from four different places in Tamil Nadu and from one place in Kerala. The RAPD-PCR was done and the amplified products were documented as in Plate 21. The primers used gave repetitive bands and non-repetitiveness was very less. The bands were scored and analyzed by NTSys Pc software, using SAHN clustering similarity index values. The dendrogram by weighted mean were generated and they were presented in Fig.1

4.4.2 Polymorphism and population differentiation

A total of 67 bands were amplified across all the five RAPD primers revealing an average of 13 bands per primer in each population (Table 16). The maximum number of

bands (27) were generated by the primer *in* 50 and the minimum number of bands (6) were generated by *in* 40.The maximum number of bands (17) were generated in the Coimbatore and Kerala populations and the minimum in the Theerthamalai (9) population (Table 17). Other populations namely Sittlingi and Iruttupallam produced 12 bands respectively. The total number of polymorphic bands (42) and monomorphic bands (25) generated by primers and the percentage of polymorphism 85.38 were recorded. The polymorphic bands, monomorphic bands generated and the percentage of polymorphism were calculated for individual primer are presented in (Table18). The per cent polymorphism ranged from 66.66 to 100 for different primers. The per cent polymorphism for certain primers (*in* 40, *in* 42) generated in the population was 100 percent. The similarity index between individual populations is presented in Table 19. The minimum similarity co-efficient (0.516) was observed between Coimbatore and Pala (Kerala) populations. The maximum similarity co- efficient was observed between Iruttupallam and Theerthamalai (0.870) populations. The similarity index values ranged from 0.580 to 0.741 in other comparisons.

4.4.3 Genetic variability of *T. iridipennis* populations explained by dendrogram

The dendrogram constructed by UPGMA method resulted in totally two clusters of *T.iridipennis* populations (A&B) out of which Pala (Kerala) formed a separate cluster (A) and other populations formed a separate cluster (B). The B had two intra clusters among which Theerthamalai,.Iruttupallam and Sittlingi formed an intra cluster and Coimbatore formed a separate intra cluster.

CHAPTER V

DISCUSSION

5.1 Nest aggregation

Nest aggregation was found to be more common in wall cavities than in tree cavities which could be attributed to the expansion of crop lands and increased felling of trees resulted in limitations in the availability of potential nesting sites. As many as 111 colonies of *T. iridipennis* were found in a dilapidated stone wall building near Sirumugai where mud was used for plastering. Cavity formation occurs too frequently whenever mud is used as a plastering material rather than cement. Similarly 84 colonies of *T. fuscobalteata* and *T. sapiens* were observed in a bamboo frame house in bamboo stem cavities (Starr and Sakagami, 1987). Keeping stingless bees in bamboo node hives is a common practice in Kerala and parts of Tamil Nadu. Likewise 16 colonies of *T. iridipennis* were found in a stone wall cavity (Swaminathan, 2000). The main reasons for congregation of nests in a site are long term availability of good nesting sites (Inoue *et al.*, 1984). Aggression between workers from rival nests and pheromone marking of potential nest sites also influence spacing between colonies (Hubbel and Johnson, 1977).

5.2 Nest architecture

The study has brought out for the first time several variations in nest architecture existing in different populations of *T. iridipennis* with respect to the presence or absence of entrance tube, size (Table 3), shape, orientation (Plate 6 to 8) and plurality of entrance tunnels (Plate 9 and 10). In many colonies of *T. iridipennis* a distinct entrance tube was found (Plate 14). The nests of natural colonies of *T. iridipennis* always had an entrance tube (Swaminathan, 2000). But unusually in one location at Anthiyur the entrance tube was surrounded by resin deposits (Plate 17) which probably serve defensive function.

Resin availability and predator pressure may be the factors responsible for such extra protective measure against the entry of pests inside the nest. In few colonies at Sittlingi and Sirumugai the entrance tube was almost absent but a circular resin ring was present around the nest entrance (Plate 13). Such a deviation in the nest entrance architecture could be attributed to the resin availability in such places. Circular nest entrance, flared up at the edges of two cm dia, due to grease deposition was reported by Swaminathan (2000). In five colonies found in one location at Yercaud hills the entrance tube was entirely absent. Instead only a small slit was found which was surrounded by a large greasy resin smear which effectively deterred the entry of ants into the nests (Plate 15). In some nest entrances the greasy resin smear turned dry due to exposure to sun and lost its ant trapping efficiency (Plate 16). The form of the nest entrance is strongly influenced by the level of exposure to wind or sun (Franck *et al.,* 2004). Resin smear around the entrance hole was found only in colonies observed in Yercaud hills. Copious availability of resin giving plants and trees in such area could be attributed for this special kind of nest entrance architecture mainly to ward of ants. Such a kind of nest entrance with a small hole surrounded by dark resin was also found in *Plebeia poecilochroa* (Drumond *et al.,* 1995).

The nest entrance architecture is highly useful to distinguish the two important genera of stingless bees. The genus *Melipona* lacks an external entrance tube and the exit is characterized by a narrow entrance hole guarded by a single guard bee. But in the genus *Trigona* usually an entrance tube is present and a number of guard bees defend the entrance (Wille and Michener, 1973). A maximum of 16 guard bees was found in one nest in the present study (Plate 4). Nest architecture is often the key feature that has been used to accord species status to morphometrically and genetically similar taxa of *Tertragonula* (Michener, 1961; Sakagami *et al.,* 1983a; Starr and Sakagami 1987; Dollin *et al.,* 1997). *T. hockingsi* nests usually lack external entrance. In one fourth of these nests nest entrances had large areas (3056 cm) daubed with globular mixtures of resin, other

materials and fresh paint collected from near by buildings. *T. carbonaria* nests normally lack external entrance tunnels. In many nests the entrances and the surrounding areas were coated with a smooth thick layer of cerumen, which may be black, red or yellow. Nests of *T. hockingsi* and *T. mellipes* are most readily distinguished by the structural characteristics of their nest (Michener, 1961; Dollin *et al.,* 1997). A dark mixture of resin and wax usually surrounds the entrance holes of *T. carbonaria* nests where as *T. hockingsi* has little or no lining at its nest entrance. Taxonomic significance of nest shape and its putative role in speciation in *T. carbonaria* and *T. hockingsi* was studied by Franck *et al.,* (2004). These species are primarily distinguished by the nest architecture, as in all other respects they are nearly identical. The entrance tube was slender and sticky in *T. moorei* (Sakagami *et al.,* 1989). In *T. cupira* the entrance was funnel shaped (Roubik, 1979). Flared up entrance was also found as an exception in *T. fuscobalteata* (Starr and Sakagami, 1987).

The diameter of the entrance tubes in different populations of *T. iridipennis* was ranging from 0.4 to 1.8 cm in the present study. Similar size variations were found to occur in different species of *Trigona.* The diameter of the entrance tube was three cm for *T. clypearis*, eight cm for *T. sapiens* and nine cm for *T. mellipes* (Dollin *et al.,* 1997). The average length of the entrance tube in different populations of *T. iridipennis* was ranging from 0.9 to 10.5 cm (Table 3). But the entrance tube was too short in *T. sapiens* (0.6 cm) and too long in *T. carbonaria* (14 cm) (Dollin *et al .,* 1997).

Out of 111 colonies observed in an old building only seven colonies had two entrances and two colonies had three entrances per nest (Table 2). All the entrances were put to active use except in one colony where one tunnel was used while the other tunnel was closed (Plate 12). Multiple entrance tunnels were occasionally observed in *T. mellipes.* There were four parallel tunnels of which two were used and remaining two were disused (Dollin *et al.,* 1997).

The entrance tube is useful to stingless bees in several ways. They build the entrance tube or select the entrance hole in such a way that it is large enough to undisturbed bee traffic and at the same time small enough to lower the predation risk. Larger colonies have larger openings that allow more bees to enter and exit simultaneously. The nest entrance is also useful to keep off ants and deny access into the nest (Sakagami *et al.,* 1983a). In *T. terminata* resin barriers and elongated entrance tubes are found to defend their nest against ants (Khoo and Young, 1987). In general the width of the opening is related to the number of bees in the colony population. In other words nest openings are neither too small to interfere with bee traffic nor so large to make defense impossible (Biesmeijer, 1999).

Variations in nest architecture was reported even between colonies belonging to the same species (Sakagami *et al.,* 1983a). Hence, it may be constituted that such variations in the nest architecture occurs also in the populations of *T. iridipennis* found in our country. Nest architecture characters were relevant but not sufficient criteria to identify species in carbonaria group. Modifications of nest architecture were probably not of prime importance in the speciation process of Australian stingless bees (Franck *et al.,* 2004). Although nest architecture has long been used to classify genera with in the Meliponini tribe (Michener, 2000). Taxonomists caution that nest morphology should be used with due care for species identification because it is strongly affected by environmental variables (Schwarz, 1948; Michener, 1961). For example open comb architecture in *T. hockingsi* may arise in response to warm temperature where as more compact form of *T. carbonaria* may arise in response to cooler temperature or because of restricted cavity size. The entrance tunnels normally built by *T. sapiens*, *T. clypearis* and *T. mellipes* are a useful diagnostic feature, but unusual local conditions can lead to aberrant entrance structures (Dollin *et al.,* 1997). *Melipona* construct nest entrances possessing external radiating ridges but in central America such structures are often lacking

(Wille and Michener, 1973). The modification of nest architecture probably result from apparently trivial mechanisms. Indeed comb structure need not necessarily be genetically determined (Franck *et al.,* 2004).

5.2.1 Nest components

Dimensions of pollen and honey pots did not differ significantly between the nest with entrance tube and nest with entrance tube surrounded by resin deposits (Table 4), the nest with circular resin ring found either in tree cavity or wall cavity (Table 8) and the nest with circular resin ring and the nest with large resin patch (Table 7). However, the dimensions of food pots and brood cells were significantly higher in nest with either circular resin ring (Table 6) or nest surrounded by large resin patch (Table 5) than nest with a normal entrance tube. Thus the present study has clearly brought out the existence of distinct variations in the size of food pots and brood cells in different populations of *T. iridipennis* building nests with and without entrance tube. The morphometric studies revealed that the bees collected from nests with entrance slit surrounded either by large resin patch or circular resin ring were lesser than bees collected from nest with an entrance tube (Table 11 and 12). Hence, the brood cells harbouring the developing bees were also proportionately larger in size in nests without an entrance tube. Since the bees were bigger in size, their food stores were also correspondingly larger in size in nests without an entrance tube. Invariably in all the nests dissected, the brood cells were arranged in clusters, a character peculiar to the bees of the genus *Trigona* (Plate 18 to 20). Similarly the queen cells were larger than the worker and drone cells, another typical character found in the genus *Trigona.*

5.3 Structural variations

Morphometric studies have indicated the existence of limited structural variations among the nine populations of *T. iridipennis* collected from different parts of India.

However, the body size variations were well pronounced in bees collected from nests without an entrance tube than from normal nests. In general the bees were bigger in size with larger body parts (Table 11 and 12). Nest entrance architecture of colonies with larger bees was markedly different from normal nests. They had relatively bigger mandibles and larger pollen baskets which were essential for resin foraging and resin transport. Resin availability in a locality also influenced the nest entrance architecture.

5.4 Genetic variations

The RAPD-PCR is an important tool for investigation of genetic polymorphism in honey bees (Hunt and Page, 1992) and stingless bees (Waldschmidt, 2000). In the present study, RAPD-PCR analysis was used to measure the genetic variations found in different populations of *T. iridipennis* collected from five different locations.

It was found that each primer generated a variable number of amplified fragments ranging from 9 to 17 (Table 17) in the five populations. These variations could be attributed the permutation combination between the DNA and primers. Absence of particular sequence in any population led to the reduction in the number of bands. The number of polymorphic markers in this study ranged from two to eight. The maximum number of polymorphic markers (8) were generated by the primer *in* 50. The minimum number of polymorphic markers (2) were produced by *in* 29. The possible cause for such variations might be either genetic drift or mutations. Waldschmidt *et al.* (2000) identified a DNA marker to differentiate the two subspecies of *Melipona quadrifasciata*. The marker was present *in Melipona quadrifasciata quadrifasciata* where as it was absent in *Melipona quadrifasciata anthidioides*, which was reflected by the presence of either continuous or discontinuous yellow stripes on the abdomen respectively.

The polymorphism in this study ranged from 66.66 to 100 per cent (Table 18) clearly indicated the existence of high degree of polymorphism which occurred due to the

heterogeneity found among different populations of *T. iridipennis*. The size variations observed among the populations and variations in nest entrance characters observed in the study could be attributed to a selection process linked or not linked to genes.

The minimum similarity index value (0.516) was observed between Coimbatore and Pala (Kerala) populations. This indicated the existence of higher heterogeneity between these two populations, which might be due to geographical isolation of these two populations, which led to the formation of two distinct clusters. Theerthamalai and Iruttupallam populations were very closely related, since the similarity index value was higher (0.87). These two populations were closely related to Sittlingi population and all the three fell in to one sub cluster. The above three locations *viz.,* Theerthamalai, Iruttupallam and Sittlingi are geographically similar and are located in the Western Ghats of Tamil Nadu.

In the present study only five primers were employed for RAPD. Precise results could be obtained only if more number of primers are used. Further SSR markers being more specific are better suited to study the population variations rather than RAPD markers. Stingless bee species specific SSR markers are better suited to study the population genetics and sociobiology of *T. carbonaria* (Green *et al.,* 2001). The present study has clearly brought out the need for considering morphometric characters, nesting behaviour and DNA data together to study the variations existing in geographically well separated populations of *T. iridipennis.*

Conclusions

The present study has clearly brought out the existence of both structural and genetic variations in the populations of *T. iridipennis* collected from different parts of India. The nest entrance architecture also varied markedly in some of the colonies collected from hill areas. It resembled the nest architecture pattern of *T. hockingsi* where

the entrance tube is entirely absent and entrance slit is surrounded by resin patch. Hence to fix the correct identity of the specimens were sent to Dr Micheal Angel, University of Kansas, Kansas. All the specimens sent were identified as *T. iridipennis* based on the morphology of worker genitalia. However, there is an urgent need to take up indepth studies to find out the biodiversity of stingless bees in India. Such a study should utilize DNA analysis using SSR markers to confirm the presence of any newer species of *Trigona* in our country.

CHAPTER VI

SUMMARY

Studies were taken up to find out the structural and genetic variations existing among the different populations of stingless bees *T. iridipennis*. The salient findings are summarized below.

- Nest aggregation was found to be more common in wall cavities than in tree cavities. As many as 111 colonies of *T. iridipennis* were found in a dilapidated stone wall building near Sirumugai.
- A prominent entrance tube of varying size, shape and orientation was found in majority of the colonies.
- The entrance tube was surrounded by dried resin deposits in one normal colony found at Anthiyur.
- The entrance tubes were almost absent and the nest entrances were surrounded by a black circular resin ring in few colonies found at Sirumugai and Sittlingi.
- A distinct entrance tube was totally absent in five colonies found inside stone wall cavities at Yercaud hills. There was only an entrance slit and was surrounded by large resinous greasy patch to ward off ants.
- Dimensions of pollen and honey pots did not differ significantly between nest with entrance tube and nest with entrance tube surrounded by resin deposits, nest with circular resin ring found either in tree cavity or wall cavity and nest with circular resin ring and nest with large resin patch.
- Dimensions of food pots and brood cells were significantly higher in nest with entrance slit surrounded by large resin patch or circular resin ring than nest with a normal entrance tube.
- Brood cells were arranged in loose clusters in all the colonies.

- Body length, antennal length and abdominal length of worker bees were similar in eight out of nine populations of *T. iridipennis* collected from nests with a distinct entrance tube from various geographical locations and only limited structural variations were found among them.
- The worker bees collected from nests with nest entrance surrounded by large resin patch and circular resin ring were larger in size than those collected from normal nest with distinct entrance tube.
- Body and mandible length of workers collected from nest with entrance slit surrounded by either large greasy resin patch or circular resin ring were greater than workers collected from nests with distinct entrance tubes.
- Nesting site (wall or tree cavity) did not influence either the nest architecture or the structural characters of the worker bees.
- The existence of genetic variations among different populations of *T. iridipennis* due to geographic isolation in India was brought out for the first time by DNA analysis using RAPD technique.
- Primers *in*40 and *in*42 are ideally suited for DNA analysis of stingless bees because they produced cent per cent polymorphism.
- Populations of *T. iridipennis* found in hilly tracts were closely related genetically since they showed higher similarity index value.

REFERENCES

Biesmeijer, K. (1993). Stingless bees: Discussions and paper at the international symposium on pollination in the tropics. *Pegone* **1**: 6-8.

Biesmeijer, K. (1999). Traits of stingless bees: 5 Nest entrances. *Pegone* **5**:9-11.

Camargo, J.M.F., Moure, J.S. and Roubik, D.W. (1988). *Melipona yucatanica*, a new species (Hymenoptera, Apidae, Meliponinae). Stingless bee dispersal across the Caribbean arc and post-eocene vicariance. *Pan. Pacif. Entomology,* **64**: 147-157.

Costa, M.A., Del Lama, M.A., Melo, G.A.R. and Sheppard, W.S. (2003). Molecular phylogeny of stingless bees (Apidae, Apinae, Meliponini) inferred from mitochondiral 16s rDNA sequence. *Apidologie* **34**: 74-93.

Darchen, R. (1970). The nests of two new species of bees from the Ivory coast, *Trigona (Axestrigona) sawadogoi* and *Trigona (Axetotrigona) eburnensis*. *Biol. Gabonica* **6**(2): 139-150.

Darchen, R. (1971). *Trigona (Axestotrigona) oyani*, a new African species of bee. Description of its nest built within an ant's nest. *Biol. Gabonica* **7**(7): 407.

De Brujin, L. (1993). Traits of stingless bees : 1 Waste dumps. *Pegone* **1**: 2-4.

DeBrujin, L. (1994). Traits of stingless bees : 2 Brood. *Pegone* **2**: 2-3.

Dollin, A.E. (1996). Nests of Australian stingless bees. Australian Native Bee Research Centre, North Richmond, 14 pp.

Dollin, A.E., Dollin, L.J. and. Sakagami, S.F. (1997). Australian stingless bees of the genus *Trigona* (Hymenoptera : Apidae). *Invertibrate Taxonomy* **11**: 861-896.

Drumond, P.M., Bego, L.S. and Melo, G.A.R. (1995). Nest architecture of the stingless bee *Plebeia poecilochora* Moure and Camargo, 1993 and related considerations (Hymenoptera. Apidae,Meliponinae). *Iheringia Serie Zoologia* **79**: 39-45.

Fletcher, D.J.C. and Crewe, R.M. (1981). Nest structure and thermoregulation in the stingless bee *Trigona (Plebeina) denoiti* Vachal (Hymenoptera: Apidae). *J. Entomol. Soc. South Afr.*, **44**(2): 183-196.

Fondrk, M.K., Page, R.E. and Hunt G.J. (1993). Paternity analysis of worker honey bees using random amplified polymorphic DNA. *Naturalissenschaften* **80**: 226-31.

Francisco, F.O., Silvertre, D. and Arias, M.C. (2001). Mitochondrial DNA characterization of five species of *Plebeia* (Apidae: Meliponini) RFLP and restriction maps. *Apidologie* **32**: 323-332.

Franck, P., Cameron, E., Good, G., Rasplus, J.Y and Oldroyd, B.P. (2004). Nest architecture and genetic differentiation in a species complex of Australian stingless bees. *Molecular Ecology* **13**: 2317-2331.

Futuyma, D.J. (1993). Biologia Evolutiva. 2nd edn. Sociedade Brasilcira de Genetica/ CNP9, Ribeirao Preto, 646 pp.

Green, C.L., Franck, P. and Oldroyd, B.P. (2001). Characterization of microsatellite loci for *Trigona carbonaria*, a stingless bee endemic to Australia. *Molecular Ecology notes* **1**: 89-92.

Harrison, R.G. (1990). Hybrid zones: Windows on evolutionary processes. *Evol. Biol.*, **7**: 129-156.

Heard, T.A. (1988). Propagation of hives of *Trigona carbonaria.* Smith (Hymenoptera: Apidae). *J.Aust.Ent.Soc.*, **27**:303-304.

Hubbell, S.P. and Johnson (1977). Competition and nest spacing in a tropical stingless bee community. *Ecology* **58**(5): 949-963.

Hunt, G.J. and Page, R.E. (1992). Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. *Theor. Appl. Genet.*, **85**: 15-20.

Hunt, G.J. and Page, R.E. (1995). Linkage map of honey bee *Apis mellifera*, based on RAPD markers. *Genetics* **139**:1371-82.

Inoue, T., Sakagami, S.F., Salmah, S. and Yamane, S. (1984). The process of colony multiplication in the Sumatran stingless bee *Trigona* (*Tetragonula*) *laeviceps. Biotropica* **16**: 100-111.

Kerr, W.E., Sakagami, S.F., Zucchi, R., Araujo V.D. and Camargo, J.M.F. (1967). Oservacoes sobre a arquiteture dos ninhose comportamento de algumas especies de abelhas sem ferrao dos vizenhancas de Manaus, *Amazonas Atas do simposio sobre a Biota Amazonica (Zool.).,* **5**: 255-309.

Khoo, S.G. and Young, H.S. (1987). Nest structure and colony defense in the stingless bee, *Trigona terminata* Smith. *Nature Malaysiana* **12**: 4-15.

Lynch, M. and Milligan, B.G. (1994). Analysis of population genetic structure with RAPD markers. *Mol. Ecol.,* **3**: 91-99.

Michener, C.D. (1961). Observations on the nests and behaviour of *Trigona* in Australia and New Guinea. *American Museum Novitaets* **2026**: 1-46.

Michener, C.D. (1964). Evolution of nests of bees. *Am. Zoologist,* **4**: 227-239.

Michener, C.D. (1974). *The social behaviour of the bees. A comparative study*. Cambridge: Harvard: University press, 404 pp.

Michener, C.D. (1990). Classification of Apidae. *University of Kansas Sciences Bulletin,* **53**: 73-153.

Michener, C.D. (2000). *The bees of the World*. Baltimore: Johns Hopkins University Press, 791 pp.

Moure, J.S. (1961). A preliminary supra-specific classification of the old world meliponinae bees (Hymenoptera, Apidae). *Studia Entomol.,* **4**: 181-242.

Nogueira-Neto, P. (1970). Acriacao de Abelhas Indigenas 5 cm Ferrao (Meliponinae). Sao Paulo, Brazil. Editora chacaras e Quintais, 365 pp.

Oldroyd, B.P., Sylvester, H.A., Wongsiri, S. and. Rindour, T.E. (1994). Task specialization in a wild bee, *Apis florea* (Hymenoptera: Apidae), revealed by RFLP banding. *Behav. Ecol. Sociobiol.*, **34**: 25-30.

Paxton, R.J., Weibschuh, N. and Quezada-Ellan, J.J.G. (1999). Characterization of dinucleotide microsatellite loci for stinglers bees. *Molecular Ecology* **8**: 685-702.

Peters, J.M., Queller, D.C., Imperatriz- Fonseca, V.L. and Straesmann, J.E. (1998). Microsatellite loci for stingless bees. *Molecular Ecology* **7**: 783-792.

Pooley, A.C and Michener, C.D. (1969). Observations on nests of stingless bees in Natal (Hymenoptera : Apidae). *J. Entomol. Soc. South Afr.*, **32**: 423-30.

Raakhee Mohan and Devanesan, S. (1999). Dammer Bee, *Trigona iridipennis* Smith (Apidae : Meliponinae) in Kerala. *Insect Environment* **5**(2): 79.

Roubik, D.W. (1979). Nest and colony characteristics of stingless bees from French Guiana (Hymenoptera : Apidae). *J. Kansas Entomol. Soc.*, **52**: 443-470.

Roubik, D.W. (1983). Nest and colony characteristics of stingless bees from Panama (Hymenoptera : Apidae). *J. Kans. Entomol. Soc.*, **56**(3): 327-355.

Roubik, D.W. (1989). Ecology and Natural History of Tropical Bees. New York: Cambridge University Press, 514 pp.

Roubik, D.W. (1992). Stingless bees : In: *Panamanian Insects.* (Quintero and Aiello, eds.). Oxford: Oxford University Press, pp. 692.

Sakagami, S.F. (1978). *Tetragonula* stingless bees of the continental Asia and Srilanka (Hymenoptera, Apidae***).*** *J. Fac. Sci. Hokkaido Univ. Ser. VI Zool.*, **31**: 165-247.

Sakagami, S.F. (1982). Stingless bees. In: *Social Insects* (H.R. Hermann, edr.). New York: Academic Press, pp. 361-423.

Sakagami, S.F., Inoue, T., Yamane, S., and Salmah, S. (1983a). Nest architecture and colony composition of the Sumatran stingless bee *Trigona (Tetragonula) laeviceps*. *Kontyu* **51**(1): 100-111.

Sakagami, S.F., Yamane, S. and Hambali, G. (1983b). Nests of some South East Asian stingless bees. *Bull. Fac. Educ. Ibraki University* (*Nat. Sci*.), **32**: 1-21.

Sakagami, S.F., Inoue, T. (1985). Taxanomic notes on three bicolour *Tetragonula* stingless bees in Southeast Asia. *Kontyu* **53**: 174-189.

Sakagami, S.F., Inoue, T., Yamane, S and Salmah, S. (1989). Nests of the myrmecophilous stingless bee, *Trigona moorei*: how do bees initiate their nest within an arboreal ant nest? *Biotropica* **21**(3): 265-274.

Schwarz, H.F. (1948). Stingless bees (Meliponidae) of the western hemisphere. *Bulletin of the American Museum of Natural History* **90**: 1-27.

Smith, F.G. (1954). Notes on the biology and waxes of four species of African *Trigona* bees (Hymenoptera: Apidae). In: *Proc.R.Ent.Soc.Lond.Ser.A.* Tabora: Bee keeping research station, **29**(46): 62-70.

Sommeijer, M.J., Houtekamer, J.L. and Bos, W. (1984). Cell construction and egg-laying in *Trigona nigra paupera* with a note on the adaptive significance of oviposition behaviour of stingless bees. *Insects Sociaux* **31**(2): 199-217.

Starr, C.K., and. Sakagami, S.F. (1987). An extraordinary concentration of stingless bee colonies in the Philippines, with notes on nest architecture (Hymenoptera : Apidae : *Trigona* spp.). *Insects Sociaux* **34**: 96-107.

Stevens, J. and Wall, R. (1995). The use of random amplified polymorphic DNA (RAPD) analysis for studies of genetic variations in populations of the blowfly *Lucilia sericata* (Diptera: Calliphoridae) in Southern England. *Bull. Entomol. Res.*, **85**: 549-555.

Suazo, A., McTiernan, R. and Hall, H.G. (1998). Differences between African and European honey bees (*Apis mellifera* L.) in random amplified polymorphic DNA (RAPD). *J. Hered.,* **89**: 32-36.

Swaminathan, T. (2000). Studies on stingless bees. M.Sc. (Ag) Thesis, submitted to Tamil Nadu Agricultural University, Coimbatore-3.

Velthuis, H.W. (1997). The biology of stingless bees. Utrecht: Department of Ethology, 33 pp.

Waldschmidt, A.M., Salomao, T.M.F., Barros, E.G. and Compos, L.A.O. (1997). Extraction of genomic DNA from *Melipona quadrifasciata* (Hymenoptera: Apidae, Meliponinae). *Braz. J. Genet.,* **20**: 421-423.

Waldschmidt, A.M., Barres, E.G. and Campos, L.A.O. (2000). A molecular marker distinguishes the subspecies *Melipona quadrifasciata quadrifasciata* and *Melipona quadrifasciata anthidiodes* (Hymenoptera: Apidae, Meliponinae). *Genet. Mol. Biol.,* **23**: 1-6.

Wille, A. (1966). Notes on two species of ground nesting stingless bees *(Trigona mirandula and T. buchwaldi*) from the pacific rain forest of Costa Rica. *Rev. Biol. Trop.,* **14**: 251-77.

Wille, A. and Michener, C.D. (1973). The nest architecture of stingless bees with special reference to those of Costa Rica (Hymenoptera, Apidae). *Rev. Biol. Trop.,* **21**: 1-278.

Wille, A. (1979). Phylogeny and relationships among the genera and subgenera of stingless bees with special reference to those of Costa Rica (Hymenoptera, Apidae). *Rev. Biol. Trop.,* **21**: 1-278.

Wille, A. (1983). Biology of the stingless bees. *Ann. Rev. Entomol.,* **28**: 41-64.

RESEARCH FINDINGS

STUDIES ON POPULATION VARIATIONS IN STINGLESS BEES (*Trigona iridipennis* Smith)

Student
Sriram Redla

Chairman
Dr. M. Muthuraman

Studies were taken up to find out the structural and genetic variations existing among the different populations of stingless bees *Trigona iridipennis*. The salient findings are summarized below.

- Nest aggregation was found to be more common in wall cavities than in tree cavities. As many as 111 colonies of *T. iridipennis* were found in a dilapidated stone wall building near Sirumugai.
- A prominent entrance tube of varying size, shape and orientation was found in majority of the colonies.
- The entrance tube was surrounded by dried resin deposits in one normal colony found at Anthiyur.
- The entrance tubes were almost absent and the nest entrances were surrounded by a black circular resin ring in few colonies found at Sirumugai and Sittlingi.
- A distinct entrance tube was totally absent in five colonies found inside stone wall cavities at Yercaud hills. There was only an entrance slit and was surrounded by large resinous greasy patch to ward off ants.
- Dimensions of pollen and honey pots did not differ significantly between nest with entrance tube and nest with entrance tube surrounded by resin deposits, nest with circular resin ring found either in tree cavity or wall cavity and nest with circular resin ring and nest with large resin patch.

- Dimensions of food pots and brood cells were significantly higher in nest with entrance slit surrounded by large resin patch or circular resin ring than nest with a normal entrance tube.
- Brood cells were arranged in loose clusters in all the colonies.
- Body length, antennal length and abdominal length of worker bees were similar in eight out of nine populations of *T. iridipennis* collected from nests with a distinct entrance tube from various geographical locations and only limited structural variations were found among them.
- The worker bees collected from nests with nest entrance surrounded by large resin patch and circular resin ring were larger in size than those collected from normal nest with distinct entrance tube.
- Body and mandible length of workers collected from nest with entrance slit surrounded by either large greasy resin patch or circular resin ring were greater than workers collected from nests with distinct entrance tubes.
- Nesting site (wall or tree cavity) did not influence either the nest architecture or the structural characters of the worker bees.
- The existence of genetic variations among different populations of *T. iridipennis* due to geographic isolation in India was brought out for the first time by DNA analysis using RAPD technique.
- Primers *in*40 and *in*42 are ideally suited for DNA analysis of stingless bees because they produced cent per cent polymorphism.
- Populations of *T. iridipennis* found in hilly tracts were closely related genetically since they showed higher similarity index value.

Table 1. RAPD primers used for DNA analysis

Primer number	Name	Sequence	Annealing temperature (°C)
in 29	ACT15’	GCTGTTTTCCCGTCCATTGT	59
in 37	Tub2i-3’	CC(AG)TG(CT)TCATCTTA(GT)AT(CT)ACCATGGA	59
in 40	Cal-1	GTGTCCTTCATTTT(AGCT)C(GT)TGCCATCAT	59
in 42	18S-S22	AG(CT)TCCATGTAGGCATTGTTGA	59
in 50	A1103	CAG TTC TT(CT) GA(GT)GCC ATA CGCT	55

Table 10. Comparison of the morphometric observations pertaining to stingless bee Workers *T. Iridipennis*

S.No	Body Character	Nest with distinct entrance tube(mm)	Nest with entrance tube surrounded by resin deposits(mm)	Significance
1	Total body length	4.55±0.06	4.85±0.90	*
2	Head length	1.75±0.02	1.88±0.44	NS
3	Head width	2.01±0.03	2.00±0.81	NS
4	Eye length	1.47±0.02	1.70±0.10	*
5	Eye width	0.39±0.01	0.36±0.59	NS
6	Inter ocellar distance	0.18±0.00	0.22±0.45	*
7	Ocello-ocular distance	0.25±0.00	0.27±0.98	NS
8	Antennal length	2.54±0.07	2.60±0.29	NS
9	Scape length	0.10±0.00	0.11±0.56	NS
10	Flagellomere-1 length	0.14±0.00	0.17±0.58	NS
11	Flagellomere-1 width	0.13±0.00	0.13±0.23	NS
12	Flagellomere-2 length	0.11±0.00	0.10±0.24	NS
13	Flagellomere-2 width	0.17±0.00	0.14±0.67	NS
14	Flagellum length	1.79±0.03	1.78±0.12	NS
15	Mandible length	0.70±0.01	0.7±0.43	NS
16	Mandible width	0.16±0.00	0.14±0.66	NS
17	Thoracic width	2.17±0.12	2.47±0.45	NS
18	Hind tibial length	1.88±0.06	1.91±0.93	NS
19	Hind tibial width	0.62±0.01	0.65±0.43	NS
20	Hind basitarsus length	0.93±0.02	1.00±0.53	NS
21	Wing diagonal	1.29±0.05	1.29±0.21	NS
22	Fore wing length	4.53±0.10	4.56±0.92	NS
23	Hind wing length	3.34±0.13	3.21±0.44	NS
24	Marginal cell length	1.58±0.03	1.59±0.80	NS
25	Marginal cell width	0.38±0.02	0.77±0.77	NS
26	Abdomen length	2.16±0.10	0.62±0.62	NS
27	Abdomen width	2.08±0.08	0.68±0.68	NS

Mean of five observations

* Differences between means are significant at 0.05 per cent by t test

NS Non Significant

Table 11. Comparison of morphometric observations pertaining to stingless bee workers *T.iridipennis*

S.No	Body Character	Nest with entrance tube (mm)	Nest entrance surrounded by large resin patch (mm)	Significance
1	Total body length	4.86±0.07	5.36±0.14	*
2	Head length	1.77±0.03	2.08±0.04	**
3	Head width	2.01±0.03	2.58±0.11	*
4	Eye length	1.59±0.04	1.65±0.02	NS
5	Eye width	0.35±0.00	0.43±0.01	*
6	Inter ocellar distance	0.22±0.01	0.17±0.00	*
7	Ocello-ocular distance	0.27±0.02	0.35±0.01	**
8	Antennal length	2.43±0.07	2.75±0.04	**
9	Scape length	0.11±0.00	0.12±0.00	NS
10	Flagellomere-1 length	0.16±0.01	0.18±0.01	NS
11	Flagellomere-1 width	0.14±0.00	0.14±0.00	NS
12	Flagellomere-2 length	0.09±0.00	0.15±0.00	**
13	Flagellomere-2 width	0.14±0.00	0.18±0.01	*
14	Flagellum length	1.56±0.06	1.82±0.03	NS
15	Mandible length	0.66±0.02	0.80±0.02	*
16	Mandible width	0.14±0.01	0.16±0.00	NS
17	Thoracic width	2.55±0.04	2.19±0.05	*
18	Hind tibial length	1.92±0.05	2.09±0.03	NS
19	Hind tibial width	0.57±0.01	0.79±0.02	**
20	Hind basitarsus length	0.96±0.03	1.12±0.02	*
21	Wing diagonal	1.22±0.04	1.68±0.09	**
22	Fore wing length	4.57±0.04	5.28±0.04	**
23	Hind wing length	3.19±0.05	3.86±0.03	**
24	Marginal cell length	1.59±0.01	1.810.03±	**
25	Marginal cell width	0.43±0.01	0.47±0.03	NS
26	Abdomen length	2.47±0.08	2.62±0.12	NS
27	Abdomen width	1.88±0.09	1.95±0.06	NS

Mean of five observations
** Differences between means are significant at 0.01 per cent by t test
* Differences between means are significant at 0.05 per cent by t test
NS Non significant

Table 12. Comparison of morphometric observations pertaining to stingless bee workers *T.iridipennis*

S.No	Body Character	Nest with entrance tube (mm)	Nest entrance surrounded by a circular resin ring (mm)	Significance
1	Total body length	4.86±0.07	5.87 ±0.22	*
2	Head length	1.77±0.03	2.58 ±0.11	**
3	Head width	2.01±0.03	2.28 ±0.01	**
4	Eye length	1.59±0.04	1.95 ±0.09	*
5	Eye width	0.35±0.00	0.39 ±0.01	NS
6	Inter ocellar distance	0.22±0.01	0.22 ±0.01	NS
7	Ocello-ocular distance	0.27±0.02	0.35 ±0.00	*
8	Antennal length	2.43±0.07	2.72 ±0.11	NS
9	Scape length	0.11±0.00	0.14 ±0.01	NS
10	Flagellomere-1 length	0.16±0.01	0.17 ±0.00	NS
11	Flagellomere-1 width	0.14±0.00	0.12 ±0.00	NS
12	Flagellomere-2 length	0.09±0.00	0.12 ±0.00	*
13	Flagellomere-2 width	0.14±0.00	0.15 ±0.00	NS
14	Flagellum length	1.56±0.06	1.78 ±0.03	*
15	Mandible length	0.66±0.02	0.69 ±0.09	NS
16	Mandible width	0.14±0.01	0.18 ±0.28	*
17	Thoracic width	2.55±0.04	2.24 ±0.77	*
18	Hind tibial length	1.92±0.05	1.98 ±0.33	NS
19	Hind tibial width	0.57±0.01	0.72 ±0.03	**
20	Hind basitarsus length	0.96±0.03	1.15 ±0.25	NS
21	Wing diagonal	1.22±0.04	1.67 ±0.00	*
22	Fore wing length	4.57±0.04	5.24 ±0.18	**
23	Hind wing length	3.19±0.05	3.90 ±0.54	*
24	Marginal cell length	1.59±0.01	1.82 ±0.53	*
25	Marginal cell width	0.43±0.01	0.42 ±0.56	NS
26	Abdomen length	2.47±0.08	3.32 ±0.93	*
27	Abdomen width	1.88±0.09	2.43 ±0.76	**

Mean of five observations

** Differences between means are significant at 0.01 per cent by t test

* Differences between means are significant at 0.05 per cent by t test

NS Non Significant

Table 13. Comparison of morphometric observations pertaining to stingless bee workers *T.iridipennis*

S.No	Body character	Nest entrance with large greasy resin patch (mm)	Nest entrance with dried resin patch (mm)	Significance
1	Total body length	5.36±0.14	4.86±0.15	*
2	Head length	2.08±0.04	2.02±0.14	NS
3	Head width	2.58±0.11	2.08±0.01	*
4	Eye length	1.65±0.02	1.46±0.07	*
5	Eye width	0.43±0.01	0.35±0.01	*
6	Inter ocellar distance	0.17±0.00	0.13±0.01	**
7	Ocello-ocular distance	0.35±0.01	0.24±0.01	*
8	Antennal length	2.75±0.04	2.54±0.05	*
9	Scape length	0.12±0.00	0.11±0.00	NS
10	Flagellomere-1 length	0.18±0.01	0.14±0.00	NS
11	Flagellomere-1 width	0.14±0.00	0.14±0.00	NS
12	Flagellomere-2 length	0.15±0.00	0.14±0.00	NS
13	Flagellomere-2 width	0.18±0.01	0.16±0.00	NS
14	Flagellum length	1.82±0.03	1.63±0.01	**
15	Mandible length	0.80±0.02	0.73±0.02	NS
16	Mandible width	0.16±0.00	0.16±0.01	NS
17	Thoracic width	2.19±0.05	2.23±0.16	NS
18	Hind tibial length	2.09±0.03	2.06±0.07	NS
19	Hind tibial width	0.79±0.02	0.71±0.01	*
20	Hind basitarsus length	1.12±0.02	1.00±0.02	*
21	Wing diagonal	1.68±0.09	1.33±0.03	*
22	Fore wing length	5.28±0.04	4.77±0.02	**
23	Hind wing length	3.86±0.03	3.30±0.03	**
24	Marginal cell length	1.810.03±	1.68±0.01	**
25	Marginal cell width	0.47±0.03	0.35±0.00	*
26	Abdomen length	2.62±0.12	2.59±0.11	NS
27	Abdomen width	1.95±0.06	1.78±0.06	NS

Mean of five observations
** Differences between means are significant at 0.01 per cent by t test
* Differences between means are significant at 0.05 per cent by t test
NS Non significant

Table 14 Comparison of morphometric observations pertaining to stingless bee workers *T.iridipennis*

S.No	Body character	Nest entrance with circular resin ring(mm)	Nest entrance with large greasy resin patch (mm)	Significance
1	Total body length	5.87 ±0.22	5.36±0.14	*
2	Head length	2.58 ±0.11	2.08±0.04	*
3	Head width	2.28 ±0.01	2.58±0.11	NS
4	Eye length	1.95 ±0.09	1.65±0.02	*
5	Eye width	0.39 ±0.01	0.43±0.01	NS
6	Inter ocellar distance	0.22 ±0.01	0.17±0.00	*
7	Ocello-ocular distance	0.35 ±0.00	0.35±0.01	NS
8	Antennal length	2.72 ±0.11	2.75±0.04	NS
9	Scape length	0.14 ±0.01	0.12±0.00	NS
10	Flagellomere-1 length	0.17 ±0.00	0.18±0.01	NS
11	Flagellomere-1 width	0.12 ±0.00	0.14±0.00	NS
12	Flagellomere-2 length	0.12 ±0.00	0.15±0.00	NS
13	Flagellomere-2 width	0.15 ±0.00	0.18±0.01	NS
14	Flagellum length	1.78 ±0.03	1.82±0.03	NS
15	Mandible length	0.69 ±0.09	0.80±0.02	*
16	Mandible width	0.18 ±0.28	0.16±0.00	NS
17	Thoracic width	2.24 ±0.77	2.19±0.05	NS
18	Hind tibial length	1.98 ±0.33	2.09±0.03	NS
19	Hind tibial width	0.72 ±0.03	0.79±0.02	NS
20	Hind basitarsus length	1.15 ±0.25	1.12±0.02	NS
21	Wing diagonal	1.67 ±0.00	1.68±0.09	NS
22	Fore wing length	5.24 ±0.18	5.28±0.04	NS
23	Hind wing length	3.90 ±0.54	3.86±0.03	NS
24	Marginal cell length	1.82 ±0.53	1.810.03±	NS
25	Marginal cell width	0.42 ±0.56	0.47±0.03	NS
26	Abdomen length	3.32 ±0.93	2.62±0.12	*
27	Abdomen width	2.43 ±0.76	1.95±0.06	*

Mean of five observations

** Differences between means are significant at 0.01 per cent by t test

* Differences between means are significant at 0.05 per cent by t test

NS Non significant

Table 15. Comparison of morphometric observations between two stingless bee Workers *T.iridipennis*

S.No	Body Character	Habitat		Significance
		Wall cavity	Tree cavity	
1	Total body length	5.81 ±0.09	5.87 ±0.22	NS
2	Head length	2.67 ±0.08	2.58 ±0.11	NS
3	Head width	2.29 ±0.12	2.28 ±0.01	NS
4	Eye length	2.09 ±0.04	1.95 ±0.09	NS
5	Eye width	0.38 ±0.01	0.39 ±0.01	NS
6	Inter ocellar distance	0.22 ±0.01	0.22 ±0.01	NS
7	Ocello-ocular distance	0.34 ±0.01	0.35 ±0.00	NS
8	Antennal length	2.95 ±0.08	2.72 ±0.11	NS
9	Scape length	0.14 ±0.00	0.14 ±0.01	NS
10	Flagellomere-1 length	0.21 ±0.00	0.17 ±0.00	**
11	Flagellomere-1 width	0.17 ±0.00	0.12 ±0.00	*
12	Flagellomere-2 length	0.14 ±0.00	0.12 ±0.00	NS
13	Flagellomere-2 width	0.20 ±0.01	0.15 ±0.00	**
14	Flagellum length	1.91 ±0.15	1.78 ±0.03	NS
15	Mandible length	0.76 ±0.02	0.69 ±0.09	NS
16	Mandible width	0.21 ±0.01	0.18 ±0.28	NS
17	Thoracic width	2.21 ±0.08	2.24 ±0.77	NS
18	Hind tibial length	2.11 ±0.12	1.98 ±0.33	NS
19	Hind tibial width	0.87 ±0.06	0.72 ±0.03	*
20	Hind basitarsus length	1.24 ±0.06	1.15 ±0.25	NS
21	Wing diagonal	1.39 ±0.02	1.67 ±0.00	**
22	Fore wing length	5.43 ±0.12	5.24 ±0.18	NS
23	Hind wing length	3.72 ±0.16	3.90 ±0.54	NS
24	Marginal cell length	1.85 ±0.02	1.82 ±0.53	NS
25	Marginal cell width	0.43 ±0.01	0.42 ±0.56	NS
26	Abdomen length	3.34 ±0.13	3.32 ±0.93	NS
27	Abdomen width	2.49 ±0.15	2.43 ±0.76	NS

\# Mean of five observations

** Differences between means are significant at 0.01 per cent by t test

* Differences between means are significant at 0.05 per cent by t test

NS Non Significant

Table 4. Comparison of nest components of stingless bee colonies *T.iridipennis*

S.No	Nest characteristics	Nest with distinct tubular entrance (mm)	Nest with tubular entrance surrounded by resin deposits(mm)	Significance
1	Honey pot length	10.8±0.59	10.4±0.56	NS
2	Honey pot width	8.3 ±0.41	8.6±0.21	NS
3	Pollen pot length	8.8 ±0.31	9.0±0.37	NS
4	Pollen pot width	7.5 ±0.20	8.0±0.29	NS
5	Larval cell length	3.8 ±0.10	3.7±0.11	NS
6	Larval cell width	3.2 ±0.11	3.2±0.88	NS
7	Cocoon length	3.9 ±0.03	3.9±0.04	NS
8	Cocoon width	2.8 ±0.10	2.9±0.03	NS

Mean of ten observations
NS Non Significant

Table 6. Comparison of the nest components of stingless bee colonies *T.iridipennis*

S.No	Nest characteristics	Nest with tubular entrance tube# (mm)	Nest with circular resin ring # (mm)	Significance
1	Honey pot length	10.8±0.59	12.2±0.84	*
2	Honey pot width	8.3 ±0.41	9.9±0.74	*
3	Pollen pot length	8.8 ±0.31	10.4±0.62	*
4	Pollen pot width	7.5 ±0.20	8.0±0.25	*
5	Larval cell length	3.8 ±0.10	4.1±0.10	**
6	Larval cell width	3.2 ±0.11	3.3±0.13	NS
7	Cocoon length	3.9 ±0.03	4.8±0.09	**
8	Cocoon width	2.8 ±0.10	3.8±0.10	**

Mean of ten observations
** Differences between means are significant at 0.01 per cent by t test
* Differences between means are significant at 0.05 per cent by t test
NS Non Significant

Table 5. Comparison of the nest components of stingless bee colonies *T.iridipennis*

S.No	Nest characteristics	Nest with tubular entrance tube# (mm)	Nest surrounded by large resin patch# (mm)	Significance
1	Honey pot length	10.8±0.59	11.1±2.06	NS
2	Honey pot width	8.3 ±0.41	9.5±0.3	*
3	Pollen pot length	8.8 ±0.31	10.0±0.26	**
4	Pollen pot width	7.5 ±0.20	9.3±0.44	**
5	Larval cell length	3.8 ±0.10	4.1±0.10	*
6	Larval cell width	3.2 ±0.11	3.1±0.11	NS
7	Cocoon length	3.9 ±0.03	4.8±0.11	**
8	Cocoon width	2.8 ±0.10	3.8±0.10	**

Mean of ten observations
** Differences between means are significant at 0.01 per cent by t test
* Differences between means are significant at 0.05 per cent by t test
NS Non Significant

Table 7. Comparison of the nest components of stingless bee colonies *T.iridipennis*

S.No	Nest characteristics	Nest with circular resin ring# (mm)	Nest surrounded by large resin patch# (mm)	Significance
1	Honey pot length	12.2±0.84	11.1±2.06	*
2	Honey pot width	9.9±0.74	9.5±0.3	NS
3	Pollen pot length	10.4±0.62	10.0±0.26	NS
4	Pollen pot width	8.0±0.25	9.3±0.44	*
5	Larval cell length	4.1±0.10	4.1±0.10	NS
6	Larval cell width	3.3±0.13	3.1±0.11	NS
7	Cocoon length	4.8±0.09	4.8±0.11	NS
8	Cocoon width	3.8±0.10	3.8±0.10	NS

Mean of ten observations
** Differences between means are significant at 0.01 per cent by t test
* Differences between means are significant at 0.05 per cent by t test
NS Non significant

Table 8. Comparison of nest components of stingless bee colonies *T.iridipennis* with resin ring collected from different habitats

S.No	Nest Character	Habitat		Significance
		Wall cavity	Tree cavity	
1	Honey pot length	12.1±0.79	12.2±0.84	NS
2	Honey pot width	9.5±0.49	9.9±0.74	NS
3	Pollen pot length	9.9±0.28	10.4±0.62	NS
4	Pollen pot width	9.2±0.37	8.0±0.25	**
5	Larval cell length	4.1±0.11	4.1±0.10	NS
6	Larval cell width	3.3±0.11	3.3±0.13	NS
7	Cocoon length	4.7±0.11	4.8±0.09	NS
8	Cocoon width	3.8±0.11	3.8±0.10	NS

Mean of ten observations
** Differences between means are significant at 0.01 per cent by t test
NS Non Significant

Table 2. Data on nest aggregation of *T. iridipennis*

Direction	Total number of colonies	Single entrance tube	Multiple entrance tubes	
			Double entrance tubes	Triple entrance tubes
North	51	51	0	0
South	26	20	5	1
East	34	31	2	1
Total	111	102	7	2

Table 3. Metric data on entrance tubes

Direction	Number of colonies	Length (cm)		Diameter (cm)	
		Range	Mean	Range	Mean
North	51	0.9-5.9	2.98	0.4-1.5	0.81
South	26	1.5-10.5	4.06	0.5-1.2	0.74
East	34	0.9-6.8	3.52	0.4-1.8	0.75

Table 9. Morphometric observations pertaining to stingless bee workers *T.iridipennis* collected from different locations (in mm)

Locations		Total body length*	Head length*	Head width*	Eye length*	Eye width*	Inter ocellar distance*	Ocello-ocular distance*	Antennal length*	Scape length*
1.	Tamil Nadu** (TNAU Campus)	4.86^{a}	1.77^{ab}	1.98^{a}	1.59^{ab}	0.35^{b}	0.22^{a}	0.27^{bc}	2.43^{ab}	0.11^{a}
2.	Malaimandiri palayam**	4.55^{ab}	1.75^{ab}	2.01^{a}	1.47^{bc}	0.39^{a}	0.18^{bc}	0.25^{cd}	2.54^{a}	0.10^{a}
3.	Yercaud**	4.56^{ab}	1.89^{ab}	2.12^{a}	1.53^{ab}	0.37^{ab}	0.16^{cd}	0.27^{bc}	2.44^{ab}	0.11^{a}
4.	Iruttupallam**	4.96^{a}	1.87^{ab}	2.03^{a}	1.63^{a}	0.39^{ab}	0.17^{cd}	0.34^{a}	2.59^{a}	0.12^{a}
5.	Theerthamalai**	4.87^{a}	1.73^{b}	1.99^{a}	1.45^{bc}	0.37^{ab}	0.22^{a}	0.27^{bc}	2.29^{b}	0.10^{a}
6.	Kerala (Pala)**	4.20^{b}	1.75^{ab}	2.04^{a}	1.36^{c}	0.36^{ab}	0.15^{d}	0.34^{a}	2.64^{a}	0.10^{a}
7.	Orissa (Bhubaneswar) **	4.70^{a}	1.82^{ab}	2.07^{a}	1.60^{ab}	0.36^{ab}	0.18^{bc}	0.30^{b}	2.59^{a}	0.10^{a}
8.	West Bengal (Birbhum)**	4.95^{a}	1.93^{a}	2.02^{a}	1.58^{ab}	0.38^{ab}	0.18^{bc}	0.23^{d}	2.51^{a}	0.10^{a}
9.	Haryana (Hissar)**	4.87^{a}	1.89^{ab}	2.07^{a}	1.60^{ab}	0.36^{ab}	0.20^{ab}	0.26^{d}	2.53^{a}	0.10^{a}

* Mean of five values

** Colonies with distinct tubular entrance

Means followed by a common letter are not significantly different at the 5% level by DMRT. (Contd.)

Table 9. Morphometric observations pertaining to stingless bee workers *T.iridipennis* collected from different locations (in mm)

Locations		Flagellomere-1 length*	Flagellomere-1 width*	Flagellomere-2 length*	Flagellomere-2 width*	Flagellum length*	Mandible length*	Mandible width*	Thoracic width*	Hind tibial length*
1.	Tamil Nadu** (TNAU Campus)	0.16^{ab}	0.14^{ab}	0.09^{cd}	0.14^{c}	1.56^{cd}	0.66^{ab}	0.14^{bc}	2.55^{a}	1.92^{b}
2.	Malaimandiri palayam**	0.14^{b}	0.13^{ab}	0.11^{abc}	0.17^{ab}	1.79^{ab}	0.70^{ab}	0.16^{ab}	2.17^{b}	1.88^{b}
3.	Yercaud**	0.13^{b}	0.13^{ab}	0.12^{a}	0.16^{bc}	1.63^{bcd}	0.69^{ab}	0.17^{ab}	1.99^{b}	1.95^{b}
4.	Iruttupallam**	0.16^{ab}	0.13^{ab}	0.10^{bc}	0.17^{ab}	1.71^{abc}	0.67^{ab}	0.13^{c}	2.21^{b}	1.97^{ab}
5.	Theerthamalai**	0.14^{b}	0.11^{ab}	0.08^{d}	0.15^{bc}	1.50^{d}	0.65^{b}	0.14^{bc}	1.99^{c}	1.94^{a}
6.	Kerala (Pala)**	0.18^{a}	0.15^{b}	0.11^{ab}	0.16^{bc}	1.85^{a}	0.72^{ab}	0.16^{abc}	1.48^{c}	2.07^{ab}
7.	Orissa (Bhubaneswar)**	0.16^{b}	0.13^{ab}	0.12^{a}	0.18^{a}	1.76^{ab}	0.75^{a}	0.15^{abc}	2.06^{b}	1.98^{ab}
8.	West Bengal (BirBhum)**	0.15^{ab}	0.12^{a}	0.10^{bc}	0.18^{a}	1.65^{bcd}	0.70^{ab}	0.15^{bc}	2.28^{b}	1.93^{b}
9.	Haryana (Hissar)**	0.15^{ab}	0.13^{ab}	0.11^{abc}	0.16^{bc}	1.74^{ab}	0.68^{ab}	0.18^{a}	2.18^{b}	1.93^{a}

* Mean of five values

** Colonies with distinct tubular entrance

Means followed by a common letter are not significantly different at the 5% level by DMRT

(Contd.)

Table 9. Morphometric observations pertaining to stingless bee workers *T.iridipennis* collected from different locations (in mm)

Locations		Hind tibial width*	Hind basitarsus length*	Wing diagonal*	Fore wing length*	Hind wing length*	Marginal cell length*	Marginal cell width*	Abdomen length*	Abdomen width*
1.	Tamil Nadu** (TNAU Campus)	0.67^{cd}	0.96^{a}	1.22^{b}	4.57^{bc}	3.19^{ab}	1.59^{b}	0.43^{a}	2.47^{ab}	1.88^{bcd}
2.	Malaimandiri palayam**	0.64^{bc}	0.93^{a}	1.29^{ab}	4.53^{c}	3.34^{ab}	1.58^{b}	0.38^{a}	2.16^{b}	2.00^{b}
3.	Yercaud**	0.70^{a}	0.95^{a}	1.45^{a}	4.77^{ad}	3.26^{ab}	1.68^{a}	0.40^{a}	2.41^{ab}	1.66^{d}
4.	Iruttupallam**	0.68^{bc}	0.99^{a}	1.41^{ab}	4.78^{a}	3.22^{ab}	1.63^{ab}	0.43^{a}	2.52^{ab}	2.02^{b}
5.	Theerthamalai**	0.69^{d}	0.94^{a}	1.32^{ab}	4.64^{abc}	3.48^{a}	1.57^{b}	0.41^{a}	2.35^{ab}	1.97^{bc}
6.	Kerala (Pala)**	0.74^{cd}	0.97^{a}	1.36^{ab}	4.66^{abc}	3.38^{a}	1.60^{b}	0.42^{a}	2.27^{ab}	1.73^{cd}
7.	Orissa (Bhubaneswar)**	0.71^{a}	0.97^{a}	1.43^{ab}	4.69^{abc}	3.05^{b}	1.60^{b}	0.37^{a}	2.58^{a}	1.77^{bcd}
8.	West Bengal (Bir Bhum)**	0.66^{ab}	0.96^{a}	1.37^{ab}	4.78^{a}	3.27^{ab}	1.64^{ab}	0.42^{a}	2.47^{ab}	2.33^{a}
9.	Haryana (Hissar)**	0.67^{ab}	0.99^{a}	1.35^{ab}	4.69^{abc}	3.42a	1.58^{b}	0.41^{a}	2.44^{ab}	2.42^{a}

* Mean of five values

** Colonies with distinct tubular entrance

Means followed by a common letter are not significantly different at the 5% level by DMRT

Table 16. Bands generated by different primers in the *T.iridipennis* populations

Primer number	Total number of bands	Polymorphic bands	Monomorphic bands	Number of amplicons	
				Min	Max
in 29	7	2	5	1	3
in 37	20	15	5	2	7
in 40	6	6	-	1	3
in 42	7	7	-	2	3
in 50	27	12	15	4	7

Table 17. RAPD bands generated by selected populations of *T.iridipennis*

S.No	Geographic places	Number of bands
1	Coimbatore (Tamil Nadu)	17
2	Sittlingi (Tamil Nadu)	12
3	Pala (Kerala)	17
4	Theerthamalai (Tamil Nadu)	9
5	Iruttupallam (Tamil Nadu)	12

Table 18. Number of markers and percent of polymorphism developed by the selected primers in *T.iridipennis* population

Primer Number	Total markers generated	Polymorphic markers	Monomorphic markers	Percent polymorphism
in 29	3	2	1	66.66
in 37	8	7	1	87.54
in 40	3	3	-	100
in 42	7	7	-	100
in 50	11	8	3	72.72

Table 19. Similarity matrix among the populations of *T.iridipennis* based on RAPD data

	Coimbatore (Tamil Nadu)	**Sittlingi (Tamil Nadu)**	**Pala (Kerala)**	**Theerthamalai (Tamil Nadu)**	**Iruttupallam (Tamil Nadu)**
Coimbatore (Tamil Nadu)	1.000				
Sittlingi (Tamil Nadu)	0.612	1.000			
Pala (Kerala	0.516	0.580	1.000		
Theerthamalai (Tamil Nadu)	0.741	0.741	0.709	1.000	
Iruttupallam (Tamil Nadu)	0.612	0.677	0.580	0.870	1.000